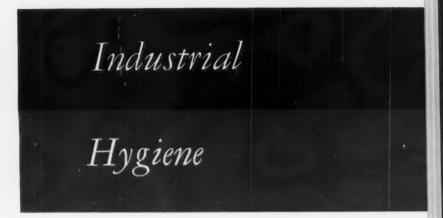
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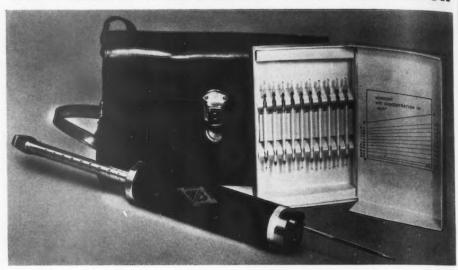
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FEBRUARY 1961

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Test Concentration mg/cu. m.	Kitagawa Results mg/cu. m.				
0.1	0.1				
0.19	0.2				
0.29	0.3				
0.41	0.4				
0.53	0.5				
0.76	0.7				
0.82	0.7				
0.84	0.8				
1.07	1.0				
1.23	1.2				
1.40	1.4				
1.53	1.5				
1.75	1.6				
1.83	1.8				
1.9	2.0-2.1				

Complete evaluation report upon request. Tubes for Measuring Atmospheric Concentrations:

Tuba No.	Detector Tube	Measurable Concentration	Tube No.	Detector Tube	Measurable Concentration
102A	Acetone-high	0.10-7.0%	124	Toluene	5-1000 PPM
102D	range	25 200 DD35	134	Trichloroethylene	10-400 PPM
102B	Acetone—low range	25—300 PPM	132	Perchloroethylene Vinyl Chloride	10-400 PPM 0.050-1.0%
101	Acetylene	100-1000 PPM	*141	Carbon Disulfide	10-200PPM
128	Acrylonitrile	.0020-5.0%	106A		25-4000 PPM
105A		1.0-25%	106C	Carbon Monoxide	25—4000 PPM
105B	Ammonia— low range	10-400 PPM		in presence of hy- drocarbons and nitrous gases	
118A		5-250 PPM	109	Chlorine	1-150 PPM
*110D	Benzene-in the pres-	10-700 PPM	116	Chlorine Dioxide	10-500 PPM
1100	ence of other aromatic	10—700 FFM	115	Cyclohexane	0.010-0.60%
	hydrocarbons		107	Diethyl Ether (Ether)	0.010-4.0%
114	Bromine	1-1600 PPM	123	Dimethyl Ether	0.010-1.5%
126A	high range	0.10-5.0%	104A		0.10-5.0%
126B	Carbon Dioxide— low range	500—12,000 PPM	111 108B	Ethyl Acetate	0.040-7.0% 0.10-50 PPM
*142	Mercury	.10-2.0 mg/M3	TUOD	range	0.10-301134
119A	Methanol-high	0.020-8.0%	122A		0.010-8.0%
129	Nickel Carbonyl	20-700 PPM	113	n-Hexane	0.010-0.60%
117	Nitrogen Dioxide	0.1-1000 PPM	112A	Hydrogen Cyanide	0.010-3.0%
121A		20-900 PPM		-high range	
	Range	,	120A		20-1700 PPM
121B	Phosphine, Low	5-90 PPM	120B	-high range Hydrogen Sulfide	1-70 PPM
103A	Range Sulfur Dioxide—	0.050-10.0%	1201	—low range	
10371	high range	0.030-10.070	120C	Hydrogen Sulfide	20-1500 PPM
103B	Sulfur Dioxide— mid range	10-1000 PPM		—in the presence of sulfur dioxide	
103C	Sulfur Dioxide— low range	5—300 PPM		tubes tested and approvers available.	ed.

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# The Respiratory Response of Guinea Pigs to the Inhalation of Acetic Acid Vapor

MARY O. AMDUR, Ph.D.

Department of Physiology, Harvard School of Public Health, Boston, Massachusetts

Guinea pigs exposed for one hour to concentrations of 5 to 500 ppm of acetic acid vapor showed an increase in pulmonary flow resistance, a decrease in pulmonary compliance, and an increase in the time constant of the lungs. These changes suggest bronchial constriction as the primary action of this irritant. Concentrations above 100 ppm also caused a decrease in respiration rate and minute volume and an increase in the work of respiration. By-passing the upper airway with a tracheal cannula increased the response. A sodium chloride aerosol delayed the recovery but did not increase the magnitude of the response.

ACETIC acid is used in the manufacture of resins and plastics, for textile processing, and in the manufacture of acetates and acetic anhydride. In lesser quantities it is used in the manufacture of pharmaceuticals, paint, and pigments, and photographic and rubber chemicals.

The vapors are irritating to the eyes and mucous membranes of the respiratory tract. Parmeggiana and Sassi' report hyperemic conjunctivae and pharyngeal mucous membranes in employees working for periods up to 12 years in atmospheres containing 26–76 ppm of acetic acid. The sunggested threshold limit for industrial atmospheres is 10 ppm.<sup>2</sup>

The present paper reports the effects of 1-hr. exposures of unanesthetized guinea pigs to acetic acid vapor at concentrations in the range of 5 to 500 ppm with the changes in mechanical behavior and ventilation of the lungs as the criteria of response.

# Methods

The physiological methods have been described in detail elsewhere. Three measurements are necessary: intrapleural pressure, tidal volume, and the rate of flow of gas in and out of the respiratory system. Intrapleural pressure was measured by recording the pressure changes in a saline-filled catheter inserted into the pleural space. Tidal volume was measured by recording the pressure changes produced in a body plethysmograph. The rate of flow of gas in the lungs and airway was measured by electrical dif-

ferentiation of the volume signal with respect to time. Simultaneous tracings of these three measurements were made on a Sanborn direct-writing oscillograph.

From measurements of these tracings flow-resistance of the lungs and airway  $(\Delta P/\Delta \dot{V})$ , cm  $\mathrm{H_2O/ml/sec}$ ) was calculated by relating the change in intrapleural pressure to the change in flow rate between inspiratory and expiratory points on the tracing corresponding to equal lung volumes. The compliance  $(\Delta V/\Delta P, \mathrm{ml/cm})$  H<sub>2</sub>O) was calculated by relating the tidal volume to the intrapleural pressure change at points of zero air flow, namely the beginning and end of inspiration.

Each animal served as its own control. The body plethysmograph was clamped so that the animal's head projected into the exposure chamber. The main air flow through the exposure chamber was started and respiratory measurements were made every five minutes for a period of half an hour. The acetic acid vapor was then added to the air stream for an hour, during which period tracings were again made every five minutes. During the post-exposure period of an hour, tracings were made every fifteen minutes.

The acetic acid vapor was generated by passing a metered air stream through glacial acetic acid. Samples for analysis were collected during each exposure period in demineralized water. The resultant conductivity of the solution was measured. From a calibration curve of the conductivity cell prepared by serial dilution of a standardized acetic acid solution and the known volume of the air sample, the concentration of acetic acid present in the exposure chamber was calculated.

This investigation was supported by a research grant (RG-4408(C5)), Division of Sanitary Engineering Services and Division of Special Health Services, Public Health Services

TABLE I Response of Guinea Pigs to One-Hour Exposure to Acetic Acid

CH <sub>2</sub> ·COOH—ppm	*******	$5.2\pm1.2$	39 ± 11	$119 \pm 8$	568 ±
Number of Animals		12	8	7	10
Body Wt.—gm		222	211	240	210
Resistance, cm H <sub>2</sub> O/ml/sec	Control	0.88	0.78	0.65	0.69
	Exposure	1.14 <sup>a</sup>	1.20a	1.43b	3,338
	% Change	+30%	+54%	+120%	+383%
Compliance, ml/cm H <sub>2</sub> O	Control	0.20	0.19	0.21	0.19
	Exposure	0.17 <sup>b</sup>	0.15°	0.15a	0.093
R × C × 10 <sup>3</sup> , min	Control	2.78	2.53	2.24	2.11
	Exposure	3.11 <sup>d</sup>	3.01 <sup>b</sup>	3.34 <sup>b</sup>	4.99
Fidal volume, ml	Control	2.14	1.54	2.24	1.61
	Exposure	2.18	1.45	2.17	1.94
Frequency, breaths/min	Control	89	100	103	109
	Exposure	80	95	83 <sup>d</sup>	56 <sup>b</sup>
Minute volume, ml/min	Control	186	159	221	177
	Exposure	170	138	176 <sup>d</sup>	109 <sup>a</sup>
Elastic work, gm-cm/min	Control	1017	624	1260	743
	Exposure	1120	667	1300	1173 <sup>b</sup>
Resistive work, gm-cm/min	Control	1311	760	1425	872
	Exposure	1425	936	1905 <sup>d</sup>	1615 <sup>b</sup>
Total work, gm-em/min	Control	2328	1384	2685	1615
	Exposure	2545	1603	3205 <sup>d</sup>	2788b

Levels of significance: p <: a -0.001 b = -0.01

c -0.02

d -0.05

#### Results and Discussion

The responses observed in guinea pigs exposed for one hour to four different concentrations of acetic acid vapor are shown in Table I. At all the concentrations studied there was an increase in the resistance accompanied by a slight decrease in compliance. The product of resistance and compliance, or the time constant of the lungs, increased during exposure at all concentrations studied. All of these changes were statistically significant as indicated in Table I.

Acetic acid vapor also produced a decrease in the respiratory rate and minute volume, but these changes were statistically significant only at the 120 and 500 ppm concentrations. From the data obtained in these studies, the elastic, resistive, and total work done in ventilating the lungs may be estimated according to formulae developed by Otis, Fenn and Rahn. The work was increased during exposure, the major increase being in the work done in overcoming resistance to air flow. These changes also were not statistically significant except at the higher concentrations used.

The time course of the responses to 568 and to 119 ppm acetic acid vapor is shown in Figure 1. In all the factors the change was greater at the higher concentration. The response commenced within the first ten minutes of exposure and in general had apparently reached its maximum at the end of 15 or 20 minutes of exposure. When the exposure was stopped the recovery commenced within the first 15 minutes. In the case of the exposure to 119 ppm the recovery was complete in an hour. In the case of the exposure to 568 ppm, some recovery was evident but the one-hour post-exposure values for resistance, compliance, time constant, frequency, and minute volume all differed significantly from the control values. Thus the methods employed in these studies are able to detect an alteration in pulmonary function at the level of a reversible response.

The respiratory response to acetic acid vapor is similar to that observed previously for sulfur

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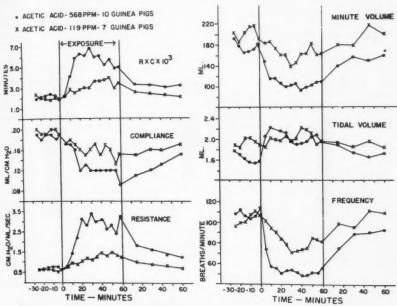


FIGURE 1. Time course of the response to two concentrations of acetic acid vapor.

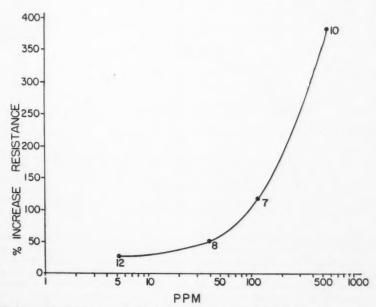
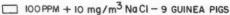


FIGURE 2. Dose-response curve for acetic acid vapor. The criterion of response is the per cent of increase over control values produced in pulmonary flow resistance by a one-hour exposure. The numbers beside each point represent the number of animals used.



## 121 PPM - TRACH. - 7 GUINEA PIGS



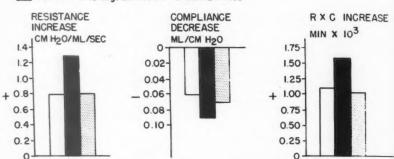


Figure 3. Comparison of response to acetic acid vapor alone breathed normally and breathed through a tracheal cannula and the response to the same concentration plus a sodium chloride aerosol.

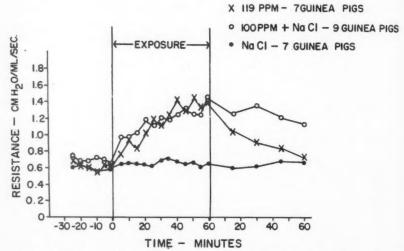


FIGURE 4. Time course of the pulmonary-flow resistance change produced by acetic acid vapor alone and plus 10 mg/m³ sodium chloride aerosol.

dioxide, formaldehyde, and formic acid. The changes observed in the mechanical behavior of the lungs (an increase in resistance accompanied by a slight decrease in compliance) suggest bronchial constriction as the primary response to the irritant. An increase in pulmonary flow resistance appears to be the most sensitive criterion so far employed for detecting the onset of a response to irritants such as these. For a given exposure time, the increase in resistance pro-

duced is related to the concentration of irritant present. Figure 2 shows the dose-response curve for acetic acid vapor. Comparison of this curve with that obtained for the other irritants studied indicates that at a given concentration acetic acid vapor produces a greater response than sulfur dioxide and a lesser response than formal-dehyde or formic acid.

The solubility of acetic acid vapor would suggest that a portion of it would be removed dur-

ing the passage of the upper airways and thus not reach the lung. By placing a cannula in the trachea and then proceeding with the routine procedure it is possible to examine the response of animals in which the protective scrubbing action of the upper airway has been eliminated. Figure 3 shows a comparison of the response of such animals with the response of normal animals to a concentration of 120 ppm acetic acid. The response was greater when the protective action of the upper respiratory tract was removed. The increase in resistance and time constant and the decrease in compliance observed in these animals was significantly greater than that observed in the normal animals at a level of p < 0.01. The protective effect of the upper airway in the case of soluble gases has been similarly demonstrated for sulfur dioxide and formaldehyde.5

Previous work has demonstrated that the responses to formaldehyde5, 6, 7 and to sulfur dioxide5, 6, 8 are potentiated by the presence of an inert aerosol. If, as suggested by LaBelle, Long, and Christofano, such potentiation is related to the amount of gas removed in the upper respiratory tract when there is no fine aerosol present to act as a carrier, one would predict that acetic acid vapor would be also potentiated by the presence of a fine aerosol. Such studies were done with a sodium chloride aerosol similar to that described previously in the studies of sulfur di-

oxide and formaldehyde.5

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Figure 3 shows that the response observed after a one-hour exposure to 100 ppm of the acetic acid vapor was the same as that observed to a similar concentration of the gas alone. In the case of formaldehyde6 the response produced by the addition of the aerosol was greater than the response produced by by-passing the upper airway. In the discussion of potentiation of the response to irritant gases by the presence of aerosols at the International Symposium on Inhaled Particles and Vapors at Oxford, Dr. La-Belle indicated that in the light of these experiments the solubility of a gas in the membranes of the upper respiratory tract does not appear to be the determining factor in whether or not a given gas is potentiated by an aerosol.

An earlier paper<sup>5</sup> indicated that the presence of an aerosol prolonged the response observed even in the case of acetic acid vapor in which

the degree of change was not altered. This was illustrated for concentrations of 30-40 ppm acetic acid alone and in the presence of the aerosol. Figure 4 indicates a similar picture for concentrations of 100-120 ppm. The animals receiving the vapor alone have returned to pre-exposure control values an hour after the exposure ended and the animals receiving the gas aerosol combination have not. In this respect the behavior of acetic and formic acids is similar.5

## Summary

Exposures of guinea pigs to vapors of acetic acid for one hour at concentrations of 5 to 500 ppm caused an increase in pulmonary flow resistance, a decrease in compliance, and an increase in the time constant of the lungs. Concentrations of 100 to 500 ppm caused a decrease in respiration rate, a decrease in minute volume and an increase in the work of respiration. The primary action is probably bronchial constriction and the over-all picture is similar to that observed for other irritant gases and vapors

Bypassing the protective action of the upper airway with a tracheal cannula increases the response to a given concentration of acetic acid vapor. The presence of a sodium chloride aerosol delays the recovery but does not increase the magnitude of the response observed.

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# A Method for Assessing the Physiological Impairment Produced by Low-level Exposure to Pulmonary Irritants

JAMES E. LONG, Sc.D., and THEODORE F. HATCH, S.M.

Department of Occupational Health, Graduate School of Public Health, University of Pittsburgh, Pennsylvania

A sensitive means is needed for discovering and measuring early and minimal degrees of pulmonary impairment following exposure to pulmonary irritants. Reduction in the rate of respiratory uptake of carbon monoxide is shown to provide such early and sensitive test. A simple apparatus and test procedure are described for measuring CO uptake in unanesthetized rats. By this method, measurable degrees of pulmonary impairment were demonstrated following short exposures (30 minutes) to phosgene in the concentration range from 5 ppm down to less than 1 ppm. Measurable reduction in CO uptake rate was found in animals, the lungs of which could not be distinguished histologically from those of unexposed animals.

#### Introduction

A NEED exists for a method capable of assessing in minimum amount the physiological impairment produced by pulmonary irritants in experimental animals, to provide a sensitive research tool for the quantitative study of relative toxicities within this important group of hazardous agents. The assessment should be in meaningful terms which measure the physiological consequences of the damage and the method should be repeatedly applicable without disturbing the animal. In this way, the time-course of development of the impairment could be followed from its beginning through the period of maximum depression to final recovery.

Pulmonary edema is the characteristic pathological state produced by irritant gases of relatively low solubility which are significantly absorbed from the inhaled air only in the lungs. The development of this condition is subtle in that it may progress to a fairly advanced state before discomfort develops or outward signs of disturbance are evident, as revealed by the common clinical indices of pulmonary performance. The desired method of assessment must be sensitive enough, however, to reveal early changes well before the development of serious physiological impairment and even before onset of subjective discomfort.

The earliest physiological consequence of pul-

monary edema is, undoubtedly, an increase in the resistance to gas diffusion across the lung membrane and, even in minimal amount, the edema must obstruct gas diffusion to some measurable degree. This concept provides the basis for the proposed test method, which, specifically, involves measurement of the rate of respiratory absorption of carbon monoxide, a gas which is uniquely dependent on the lung diffusion capacity for its uptake rate. Respiratory absorption of CO is a function of both the rate of effective lung ventilation and pulmonary diffusion capacity, the two operating in series. It is independent of pulmonary blood flow.1 In the healthy animal at rest the capacity for transporting CO across the lung wall by diffusion, is considerably greater than the rate of delivery of the gas into the lungs by ventilation, so that it is the latter which largely determines the CO uptake rate. With the onset of pulmonary edema, however, the reduced diffusion capacity becomes relatively more important and will, when sufficiently lowered, become the limiting factor determining CO uptake rate. Thus, measurements of CO uptake rate before and successively in time after exposure of an animal to a pulmonary irritant gas should provide a quantitative method for assessing the degree of physiological impairment and the time-course of development and recovery from the consequent edema. A method for carrying out such measurements and an evaluation of the procedure is presented in this paper.

The work reported in this paper was done under National Institutes of Health Grant S-27, The Influence of Air Pollutants on Cardiorespiratory Performance.

# **Experimental Apparatus and Procedure**

A closed-circuit respirometer is employed (Figure 1) consisting of an animal chamber (526 ml), an air circulating pump (AC Fuel Pump), an absorbent chamber containing drierite and ascarite for removing expired water vapor and carbon dioxide, and a thermal equilibrating coil. These are connected in series in a closed circuit by means of copper tubing and all immersed in a water bath. The pump circulates the air within the system at approximately 8 liters/min. to insure complete and continuous mixing. A branching tube from the closed circuit leads through a flowmeter into the 50 ml ionization chamber of a vibrating-reed electrometer (Applied Physics Corp.) and back into the main circuit. By means of a valve in the main line, the flow rate through the ionization chamber is adjusted to 1.5 liters/min. to insure a high rate of sample replacement in the analyzer. Using C"O as the test gas, this arrangement for rapid sampling and analysis provides continuous measurement and recording of the CO concentration in the circulating air.

An unanesthetized rat is placed inside the animal chamber, the system sealed and the aircirculating pump started. The electronic system is next balanced to give a zero reading and then one ml of carbon monoxide (mixture of normal CO and C4O) is introduced through port B. The test gas quickly mixes throughout the volume of circulating air and its concentration starts at once to drop in proportion to its rate of respiratory absorption by the animal. The initial CO concentration is about 1200 ppm which is well within physiological tolerance limits for a ten minute exposure. Over this short test period only a minute quantity is absorbed by the animal so that the blood concentration remains essentially zero. Under these conditions, with a reasonably steady volume and frequency of breathing, the rate of CO absorption per unit concentration in the inhaled air will remain constant. Hence, there will be a constant percentage decrease in the CO concentration in the recirculating air. A plot of concentration versus time will therefore give a straight line on semilogarithmic paper, the negative slope K of which will be directly proportional to the overall coefficient of respiratory uptake,  $U_{co}$  (ml/min), and inversely proportional to the volume, W in ml, of the recirculating air stream:

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$$K = \frac{U_{\text{CO}}}{W}$$

The usual test period is eight to ten minutes. From the resulting record, ratios of the scale

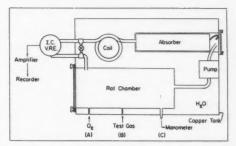


FIGURE 1. Respiratory function apparatus.

reading at time T ( $C_{\tau}$ ) to the initial reading at time zero ( $C_{\circ}$ ) are calculated for half-minute intervals and plotted versus time in minutes on semi-logarithmic graph paper. From the visually fitted straight line, the ten-minute  $C_{\tau}/C_{\circ}$  ratio is obtained, from which the slope, K, of the line is calculated:

$$K = \frac{2.3 \log \frac{1}{C_T/C_o}}{T}$$

where, K = slope of line

C =concentration (scale reading)

T = time in minutes

An interval of one hour between tests provides enough time for elimination of carbon monoxide-C<sup>14</sup> down to an insignificant level at the beginning of the next test. This was demonstrated by the fact that, after this interval, no measurable quantity of carbon monoxide was given off by the animal when tested in the respirometer.

# Additional Measurements with Respirometer

A water-filled manometer connected to port C on the respirometer indicates the system pressure. It also provides a means for estimating the breathing frequency of the rat since with each breath there is a detectable movement of the water column. By counting the frequency every other minute during the test and taking the average, an approximate normal respiration frequency for a given rat is obtained.

During a test the animal takes up oxygen from the circulating air. Exhaled CO<sub>2</sub> and water vapor, on the other hand, are continuously removed in the absorbing chamber. In consequence, there is a tendency for the pressure within the system to go down. To offset this, oxygen is drawn from an inverted 100-ml syringe connected to port A and the quantity withdrawn is shown by the volume of water which replaces

it in the syringe. In this way, the rate of oxygen consumption by the animal is estimated.

With this test procedure each rat serves as his own control. Hence, a minimum of two or three pre-exposure tests are made on each animal, depending on the variation, to provide individual base-line values representing the normal carbon monoxide uptake rate, breathing frequency, and oxygen consumption, against which to compare post-exposure rates.

## Normal CO Uptake

Twenty-five male rats (Wistar strain, 240-290 gm body weight) were randomly selected from the colony for measurement of "normal" CO uptake rate in apparently healthy animals. At least two ten minute runs were conducted on each rat. The results were examined in terms of the ratio of CO concentration after ten minutes to the initial concentration. This ratio,  $C_r/C_o$ , is related to the carbon monoxide uptake rate,  $U_{\rm co}$ , as follows:  $\log C_{\rm r}/C_{\rm o} = -4.343~U_{\rm co}/{\rm W}$ , and, since W was kept essentially constant by using animals of approximately equal weights, the ratio  $C_r/C_o$  served as a convenient index of uptake rate. The mean value of  $C_r/C_o$  for the fifty measurements was 0.41; hence, with an average animal weight of 250 grams, the average value of  $U_{co}$  was about 53 ml/min. The breathing volume of a 250 gram rat at rest is around 100 ml/min.2 Thus, the percentage carbon monoxide retention in the average test was about 53 per

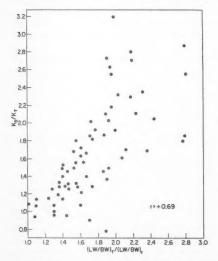


FIGURE 2. Carbon monoxide uptake versus lungto-body weight ratio for the 66 Antu-treated rats.

cent, which is in agreement with commonly reported values for humans.3

The  $C_{\rm T}/C_{\rm o}$  ratio ranged among the 25 normal animals from 0.34 to 0.52 and the maximum difference between tests on a single animal was 0.09. With nine of the animals, the test-to-test difference did not exceed 0.01. This rather narrow range of variability in the data was confirmed by the results of a one-way classification analysis. The standard deviation of the test-to-test variation on a given rat,  $\sigma_{\rm e}=0.027$ , was smaller than the standard deviation between rats,  $\sigma_{\rm m}=0.035$ . In both cases they were substantially less than 10 per cent of the mean value to  $C_{\rm T}/C_{\rm o}$  and, similarly, represent a difference of no more than ten per cent of the carbon monoxide uptake rates.

## CO Uptake vs. Lung Weight/Body Weight

To validate the change in carbon monoxide uptake as an index of pulmonary edema, it was necessary to demonstrate a systematic relationship with another independent assessment of the magnitude of edema. Based on previous studies on mice, an increase in the ratio of lung to body weight (LW/BW) was chosen as such a separate index, the rationale being that with the accumulation of edema fluid in the lungs, this ratio must increase.

Fifteen male rats (239–287 grams) were weighed and sacrificed. The lungs were dissected out, extraneous material trimmed off, and trachea cut to a specified constant length. The prepared lung was weighed and ratio to body weight calculated. The lung-to-body weight ratios (LW/BW) for these rats fell within the relatively narrow range of 0.00395 to 0.00433, giving a mean of 0.00414 and standard deviation of 0.0000186 or 4.5% of the mean. The findings indicate considerable stability in the LW/BW ratio in a normal group of stock animals. The average ratio of 0.00414 from these measurements was used as a base-line value for comparison with ratios found in edematous animals.

To determine the correlation between lung-body weight ratio and carbon monoxide uptake rate, sixty-six previously weighed rats were dose with Antu by stomach tube at the level of 28.6 micrograms per kg body weight. At hourly intervals, several rats were tested in the respirometer and then sacrificed. The lungs were dissected out, trimmed, weighed and the LW/BW determined. The ratios of LW/BW at time T after exposure to the normal LW/BW (average value given above) and the comparison ratios of the normal carbon monoxide uptake to that at time T were calculated. A plot of these two indices is shown in Figure 2.

The correlation coefficient of +0.69 suggests only a moderate association between the two measures of edema. One possible explanation for this relatively low correlation could be a nonuniform distribution of the edema in the lungs. Thus, two animals could have the same LW/BW ratio but, with different patterns of fluid distribution, these equal quantities of fluids could be accompanied by quite different disturbances in carbon monoxide uptake. Similarly, with respect to CO uptake as an index of edema, there is possibility of error, owing to the fact that the carbon monoxide absorption is a function of ventilation rate and efficiency, as well as diffusion capacity. Again, unequal distribution of fluids in the lungs could affect these two components of the gas-exchange process to entirely different degrees. Despite the considerable scatter seen in Figure 2, and the foregoing comments, it is evident that the loss in CO uptake capacity and the increase in fluid content of the lungs do progress in parallel as edema develops and the findings do support the usefulness of CO uptake as a measure of the magnitude of the edema.

## Time-Patterns of Pulmonary Edema

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Eight rats were administered a sublethal dose of Antu  $(28.6\gamma/kg)$  via stomach tube and tested at hourly intervals up to 8 hours and at 24, 48 and 72 hours, to measure the changes in carbon monoxide uptake, oxygen consumption and breathing frequency over the entire cycle of depression and recovery from the edema. Two of

the eight animals died six and nine hours after treatment. The other six apparently recovered fully since, at 72 hours, their various indices of respiratory performance had returned approximately to pre-treated levels.

Tests were also conducted with phosgene to provide a time-pattern of response from a gaseous deep-lung irritant taken in by respiration for comparison with the Antu-induced pattern (oral administration of agent). Seven male rats were exposed in these tests to 10 ppm for 15 minutes.

The data were examined in terms of ratios of the normal CO uptake and oxygen consumption to the rates at time T after exposure and typical results are shown in Figures 3 and 4.

These graphs show evidence of progressive deterioration, beginning within the first hour after treatment and advancing to a level of maximum depression between the fifth and eighth hours. Partial recovery was exhibited after 48 hours and recovery was apparently complete within three days. The degree of depression was greater for carbon monoxide uptake than for oxygen consumption. Breathing frequency was not changed significantly. Figures 3 and 4 show essentially the same time patterns, suggesting that the history of development and recovery from the edema is independent of the route of administration of the agent.

It is evident from the graphs that there is no delay in the onset of edema since measurable changes in CO uptake rate were noted within one hour after the insult. This supports the view

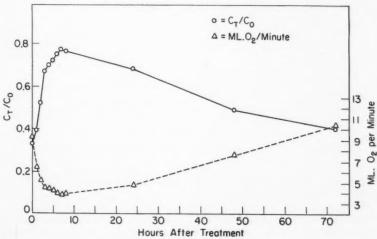


FIGURE 3. Changes in carbon monoxide uptake and oxygen consumption with time after treatment with Antu (28.6 $\gamma$ /kg body weight).

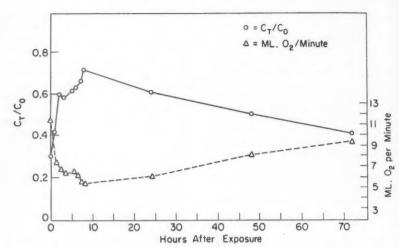


FIGURE 4. Changes in carbon monoxide uptake and oxygen consumption with time after exposure to 10 ppm, phosgene for 10 minutes.

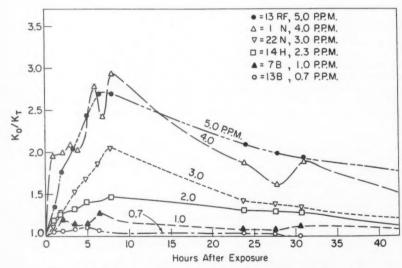


FIGURE 5. Carbon monoxide uptake in rats after exposure to phosgene.

that the characteristic lag of several hours between exposure to pulmonary irritant gases and onset of distress reflects the time required to build up sufficient amount of edema to produce the ill-effect rather than a delay in the irritant action, itself, between the chemical agent and the lung tissue.

# Low Levels of Phosgene

Findings from the preliminary studies described above indicated that the proposed test method is readily applicable to small animals and is capable of revealing physiological changes occurring in the early stages of pulmonary edema. The next and final step was to demonstrate the contract of the present that the proposed in the preliminary studies described in the proposed test method is readily applicable to small animals and is capable of revealing physiological changes occurring in the early stages of pulmonary edema.

strate its practical usefulness as a sensitive research tool for assessing the physiological changes resulting from exposure to pulmonary irritants in low and "realistic" concentrations.

The experimental plan called for at least eight rats to be exposed to each of the following concentration ranges for 30 minutes: 0.5-1; 1-2; 2-3; 3-4; and 4-5 ppm phosgene. Baseline averages for carbon monoxide uptake, oxygen consumption and breathing frequency were obtained for each of the 49 rats used in the study, after which 30 minute exposures were conducted.

Starting at one hour after the termination of the exposure, post exposure tests were run at 1, 2, 3½, 5, 6½, 8, 24, 28, 31, 48 and 72 hours. The carbon monoxide retention, oxygen consumption and breathing frequency were measured in each test.

Changes in capacity to absorb carbon monoxide following exposures to various concentrations of phosgene ranging between 0.7 and 5.0 ppm are shown  $(K_{\circ}/K_{\tau} \text{ ratios})$  in Figure 5. These are typical examples from the total of 49 such graphs obtained. The illustrative graphs all exhibit the same pattern of progressive loss of capacity to absorb CO over the first six to eight hours after exposure followed by a prolonged period of recovery. Of special interest is the evidence of a systematic decrease in the magnitude of the effect in parallel with the lowering of the phosgene concentration and of even greater interest is the fact that measurable changes were found with the lowest levels of exposure, below one ppm. In order to assign a value of relative physio-

logical cost to the overall experience in each

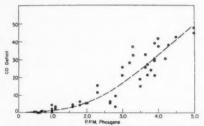


FIGURE 6. CO deficit versus phosgene concentration for 49 rats exposed to various levels (0.5-5.0 ppm).

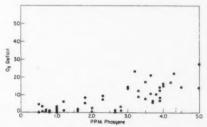


FIGURE 8. Oxygen deficit versus phosgene concentration for 49 rats exposed to various levels (0.5-5.0 ppm).

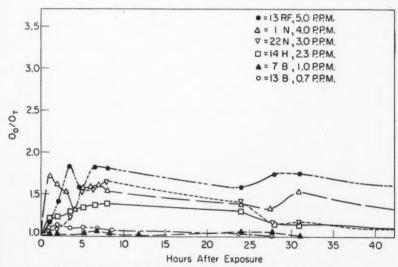


Figure 7. Oxygen consumption in rats after exposure to phosgene.

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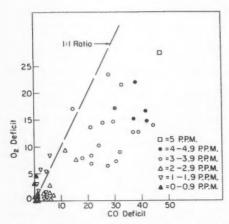


FIGURE 9. Oxygen deficit versus carbon monoxide deficit for the 49 phosgene exposure rats.

case, from early minimal to maximal changes and back to normal again, the areas under the curves were determined for each of the 49 rats and these areas denoted as CO DEFICIT values. In each case, they are proportional to the accumulated loss of capacity (relative to the normal pre-exposure capacity) to absorb carbon monoxide throughout the whole period of depression, from the onset of edema to recovery.

The estimates of the CO deficit, obtained in this manner, are plotted against the corresponding exposure levels of phosgene in Figure 6. Each animal is represented by a separate point. Although there is considerable scatter in the results, a definite relationship is exhibited between the level of exposure and physiological effect,

with a suggested break in the dose-response pattern at about 2.0 ppm.

Similar analyses were made in respect to the cumulative loss in oxygen consumption. Time plots of oxygen consumption for various exposure levels are shown in Figure 7 and O<sub>2</sub>-deficit values are plotted against exposure concentrations in Figure 8.

It is to be noted, by comparing Figures 6 and 8, that the magnitude of the effect of the pulmonary edema upon oxygen transport into the blood was not as great as its interference with CO uptake. Also, there is more scatter, suggesting that the oxygen uptake is not so completely dependent upon the specific lung impairment. This is shown more clearly in Figure 9, which presents a plot of CO versus O<sub>2</sub> deficit. The ratio of deficits is not 1:1 as it would be if the uptake rates for the two gases had been equally affected.

There is a reasonable explanation for the greater sensitivity of carbon monoxide uptake as a measure of pulmonary edema. As noted earlier, CO uptake depends upon the magnitude and efficiency of ventilation operating in series with the diffusion capacity but is independent of the rate of blood flow, whereas, for oxygen, the exchange rate depends upon blood flow as well as the other two components of the cardiorespiratory system. From this it follows that, to whatever degree there is an increase in pulmonary blood flow in an effort to compensate for respiratory impairment, this increase will be of benefit in respect to oxygen transfer but, will not affect earbon monoxide uptake.

Figure 10 shows that breathing frequency was not significantly changed and this must be regarded as an insensitive index of impairment.

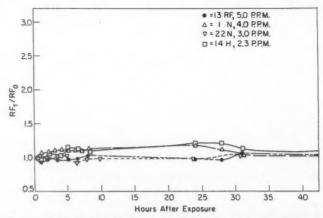


FIGURE 10. Respiration frequency in rats after exposure to phosgene.

# CO Uptake vs. Histology of Lung

Additional rats were included in several of the low-level exposures for histological examination and correlation of microscopic assessment with changes in carbon monoxide uptake in companion animals. These animals were killed seven hours after exposure and the lungs dissected out, fixed, sectioned and stained with hematoxylin and eosin. The slides were rated on the basis of two categories: (1) the extent (spread) of the edema, and (2) the quantity of hemorrhage and congestion present. The rating scales utilized were:

#### Edema

0 no edema

± a few areas of pink staining

+ approximately 10% of alveoli affected ++ approximately 30% of alveoli affected

+++ approximately 66% of alveoli affected ++++ greater than an approximate 66% affected

#### Hemorrhage

0 normal

+ slight excess over normal amount

++ quite extended

+++ severe, diffused.

The results of such semi-quantitative assessment of microscopic changes seen in the lung sections showed a rough correlation with the magnitude of loss in capacity to absorb CO observed in companion animals, but the agreement was best for the higher exposure levels which produced considerable lung damage. The lungs of animals exposed up to 1.3 ppm were not clearly separated by histological assessment from those of normal animals, whereas there was significant difference in terms of reduced capacity to absorb carbon monoxide. This suggests that the change in CO uptake provides a more sensitive index of beginning impairment. To this we can add the advantage, noted earlier, that the test can be applied repeatedly, thus making it possi-

ble to follow the development of and recovery from the edema in the same animal.

#### Summary

- A new method is offered as a research tool for study of the toxic effects of pulmonary irritant gases.
- 2. The advantages of the method are:
  - (a) It provides a direct and dynamic measure of the changes in functional capacity of the respiratory system to exchange gases and thus measures edema in terms of its major physiologic consequence.
  - (b) It gives quantitative measurements with high stability and sensitivity. Sensitivity is indicated by the fact that significant changes in pulmonary performance were observed following a 30 minute exposure to less than one ppm of phosgene.
  - (c) It does not disturb the test animal so that repeated measurements can be made at hourly intervals, thus making it possible to follow the respiratory changes in time and to portray the experiences of animals over periods of continuing exposures as well as after single exposures.

## Acknowledgments

We are indebted to Drs. Paul Gross and Mario Battigelli for their histological evaluations.

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# To OUR AUTHORS AND READERS

WITH THIS ISSUE OF THE AIHA JOURNAL we initiate our newly adopted policy of carrying an abstract of each article at the head of the article. This change in our format has been suggested and requested from many quarters and we hope that you find it useful and worthwhile. Your comments will be appreciated.

This does add one more burden on our authors in that they now are asked to supply the abstract for this purpose. Dear authors, we shall appreciate your cooperation in this respect. The abstracts should be brief, preferably about 100 words or less, and should point out the major work or results being reported. Please include such an abstract with all manuscripts submitted in the future.

# The Acute Inhalation Toxicity of Heated Aerosols of Three Hydraulic Fluids

LEO FEINSILVER, JOSEPH PERREGRINO, MARTIN A. ROSS, PAUL P. YEVICH, ROBERT M. STOUFER, and FRED W. OBERST

Directorate of Medical Research, Army Chemical Center, Maryland

A comparative study was made of the effect of pyrolysis at various temperatures upon the toxicity of aerosols of three aircraft engine hydraulic fluids. Rats and dogs were exposed 4 hours to both unpyrolyzed aerosols and aerosols pyrolyzed at 520°, 1020°, and 1560°F of the following fluids: OS-40, a phosphate ester; XF-408, a mixture of a silicone oil and a fluorohydrocarbon; and MLO-5277, an organosilicate. The toxic signs and pathologic changes produced in exposed animals showed that these compounds and their pyrolytic products were eye and respiratory irritants. OS-40 was the least toxic of the three fluids.

#### Introduction

STUDY of the behavior of certain organic compounds which come in contact with heated metal surfaces has recently become important due to the development and use of new and more powerful engines operating at higher temperatures. It is conceivable that, under conditions of high temperature operation, toxic products may be generated by pyrolysis of lubricants and hydraulic fluids coming in contact with hot metal. This laboratory recently completed investigations on the acute inhalation toxicity of the air-borne products resulting from subjecting aerosols of several engine lubricating oils to pyrolysis at temperatures of 400° and 700°F.1 Similar studies have been carried out by Treon and his collaborators.2, 3, 4, 5 As a continuation of previous work in the laboratory, this paper describes the acute toxic effects in animals inhaling the air-borne products resulting from subjecting aerosols of three hydraulic fluids to pyrolysis at temperatures of 520°, 1020°, and 1560°F. These products will hereinafter be referred to as the "pyrolyzed" fluid, for the sake of brevity.

#### Methods

Dispersion and Heating of the Hydraulic Fluids

The following hydraulic fluids were tested: OS-40, a phosphate ester, XF-408, a mixture of a silicone oil and a fluorochlorohydrocarbon, and MLO-5277, an organo-silicate.

A diagram of the apparatus used for the dispersion and heating of the hydraulic fluids is shown in Figure 1. The fluids were dispersed

as aerosols by passing air through a submerged Laskin aerosol generator. The total air flow through the apparatus was kept constant at a rate of 33 liters per minute. A feed reservoir was attached to the dispersion vessel to maintain a constant level of fluid at the base of the generator.

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Nominal concentration (mg/liter) of a hydraulic fluid in the chamber was calculated by dividing the weight (mg) of a fluid dispersed by the total volume of air (liters) required to disperse the fluid. Variations in concentration were effected by varying the air pressure (2.5-20 psig) to the dispersion generator. The feed rate of a hydraulic fluid was calculated by dividing the weight of the fluid dispersed by the total time of dispersion.

The aerosol was passed first through an alloy steel (AMS 5591 stainless) pyrolysis tube loosely packed with turnings of identical composition. The tube was heated in a Lindbergh electric furnace. The furnace pyrometer was calibrated with an independent pyrometer, which was connected to a thermocouple inserted into the heated and packed tube through which air flowed at 33 liters per minute. A temperature gradient was present in the pyrolysis tube and the maximum observed temperature in the tube was considerd to be the temperature of pyrolysis.

The aerosolized oil with its pyrolysis products was cooled by passage through a heat exchanger. The mixture next entered a glass air-mixing cylinder inserted into a constant flow gassing chamber containing animals. By this combined means

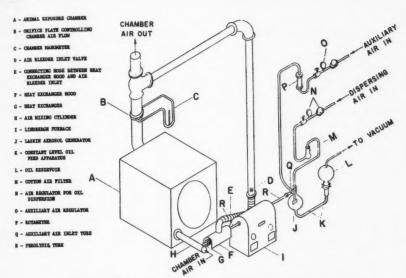


FIGURE 1. Apparatus for dispersion and pyrolysis of hydraulic fluids.

of cooling it was possible to keep the temperature of the chamber air in the range of 70 to 86°F. The capacity of the chamber was 700 liters and the air flow through it was 105 liters per minute.

When MLO-5277 was heated, no turnings were used in the pyrolysis tube, because this fluid decomposed at temperatures as low as 520°F, and the resulting fine powder (silica?) blocked the flow of air. To compensate for the reduction in surface caused by absence of the packing, a steel spiral (A.I.S.I. 304 stainless) was inserted into the pyrolysis tube and was rotated continuously at a speed of 4 rpm. It was shown by control experiments that pyrolysis under these conditions produced the same effects in animals as pyrolysis in the packed tube.

#### Analysis of Chamber Air

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Several thermal decomposition products of each fluid were selected for quantitative determination in the chamber air. Those from OS-40 were analyzed for: (a) inorganic phosphate, determined colorimetrically as the molybdivanado-complex<sup>7</sup> and calculated as phosphoric acid; (b) phenols, determined by bromination<sup>8</sup> and calculated as cresol; (c) total aldehydes determined iodimetrically<sup>9</sup> and expressed as formaldehyde; and (d) carbon monoxide, estimated by the MSA carbon monoxide detector.<sup>10</sup>

Pyrolysis products of XF-408 were analyzed for (a) inorganic chloride, determined by a

modification of the Volhard procedure,<sup>31</sup> and expressed as hydrogen chloride; (b) inorganic fluoride, determined by the thorium nitrate titration<sup>32</sup> and expressed as hydrogen fluoride; and (c) total aldehydes and carbon monoxide, determined by the same methods as those used for OS-40. In several instances, the carbon monoxide content of the blood of dogs exposed to pyrolyzed XF-408 was determined by the micro-procedure of Scholander and Roughton.<sup>33</sup>

Pyrolysis products of MLO-5277 were analyzed for carbon monoxide and total aldehydes as described above.

In addition to the above chemical determinations, the total particulate matter was determined by weighing the material collected by passing a known volume of chamber air through an aerosol collector.

# Exposure of Experimental Animals

Male and female white rats, ranging in weight from 230 to 400 gm, and male and female mongrel dogs ranging in weight from 15 to 20 lbs, were used. The rats were of a derived Wistar strain bred in the Chemical Warfare Laboratories colony. All animals were exposed once for 4 hours to the air-borne products resulting from spraying aerosols of each hydraulic fluid through the pyrolysis tube which was maintained at temperatures of 69–80°, 520°, 1020°, or 1560°F. Nominal concentrations ranged from 0.3 to 4.4 mg/liter. Fluid feed rates ranged from 30–535

mg/min. Rats were exposed in two groups of 10 each. In one group animals were killed at intervals after exposure and examined for changes in the lung, trachea, heart, kidney, liver, spleen, stomach, intestine, bladder, and genitalia. In the other group seven days after exposure, the cumulative mortality was noted and pathologic changes in survivors were studied. Dogs were exposed in groups of two each, and were sacrificed at irregular intervals after exposure, depending on their clinical condition. The lungs, trachea, heart, liver, kidney, spleen, and occasionally the genitalia were examined.

#### Results

## OS-40

Chamber air analyses and mortalities in each species are shown in Table I. No toxic signs were observed in rats exposed to the highest concentration of unheated OS-40 (4.3 mg/liter). Rats exposed to similar concentrations (3.5 to 3.9 mg/liter) of OS-40 pyrolyzed at 520° and 1020° showed a slight reddish secretion about the eyes and nose. Rats exposed to products of pyrolysis at 1560° showed a similar but more profuse seretion and severe respiratory distress. At 1560° there was 80% mortality compared with no deaths at the other temperatures.

Rats exposed to unheated OS-40 and to OS-40 pyrolyzed at 520° and 1020° did not show any pathologic changes. When the aerosol was heated at 1560°, there was severe damage to the respiratory tract. This was characterized by necrosis of the epithelium of the upper air passages, tra-

chea, and larger bronchi with formation of a diphtheritic-like membrane, and massive hemorrhage and edema of the lungs. No changes were seen in the other organs examined.

Toxic signs in dogs exposed to unheated OS-40 (4.3 mg/liter) and OS-40 pyrolyzed at 520° and at 1020° (3.5 to 3.9 mg/liter) were salivation, rhinitis, and conjunctivitis. Exposure to the 1560°-pyrolysis products increased the severity of these signs and, in addition, caused severe respiratory distress with moist rales.

Pathologic changes in dogs exposed to 08-40 pyrolyzed at 1560° were similar to those seen in rats, i.e., severe damage to the respiratory tract. At the lower pyrolysis temperatures there were corresponding decreases in tissue damage. Products of pyrolysis at 1020° caused moderate degeneration of bronchiolar epithelium with rapid recovery several days following exposure. Products of pyrolysis at 520° caused an increase in activity of mucous cells, without evidence of tissue destruction. No changes were seen in dogs exposed to unheated aerosols.

# XF-408

Chamber air analyses and mortalities in each species are shown in Table II. The only toxic sign in rats exposed to unheated XF-408 was eye irritation, except that at the highest concentration (4.2 mg/liter) respiratory distress was also present. As the pyrolysis temperature was increased, increased severity of respiratory distress and eye irritation was noted. Products from 1560° pyrolysis caused 100% mortality at concentrations as low as 1.2 mg/liter; comparable

Table I

Toxicity to Rats and Dogs of Heated Aerosols of Hydraulic Fluid OS-40

Exposure period: 4 hours

	Fluid feed	Nominal fluid		Ch	amber ana	lyses		Mortality fraction	Time of sacrifice
Tube temp	rate	concn	TPM*	Free phos- phoric acid	Phenols	Carbon monoxide	Aldehydes	rats	dogs
°F	mg/min	mg/l	mg/l	mg/l	mg/l	ppm	ppm		
75	451	4.30	2.28	0.00	0.06	Negative	2	0/10	1/2 at 4th day 1/2 at 7th day
520	362	3.45	1.47	0.04	0.14	10	1	0/10	2/2 at 6th day
1020	374	3.56	1.30	0.06	0.19	25	4	0/10	1/2 at 2nd day 1/2 at 7th day
1560	410	3.90	0.80	0.18	0.08	1700	95	8/10	1/2 at 2nd day 1/2 at 3rd day

<sup>\*</sup> TPM = Total particulate matter.

<sup>\*\*</sup> Time of sacrifice refers to period following removal from chamber.

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Table II

Toxicity to Rats and Dogs of Heated Aerosols of Hydraulic Fluid XF-408

Exposure period: 4 hours

m 1	Fluid	Nomi- nal	Chamber analyses					Mortality fraction	Time of sacrifice
Tube temp	feed	fluid	ТРМ	HCI	HF	Alde- hydes	Carbon monoxide	rats	dogs
°F	mg/ min	mg/l	mg/l	ppm	ppm	ppm	ppm		
76	205	1.69	0.57	-	-		_	-	2/2 at 13th day.
80	229	1.89	0.57	0.5	2.1	Trace	Negative	0/10	1/2 at 14th day; 1/2 observed.
71	350	2.89	1.06		-	-	-	0/20	
74	356	2.94	1.12	-	-	-	-	-	1/2 at 7th day; 1/2 at 8th day.
79	507	4.19	1.37	2.2	0.2	0.5	-	8/10	1/2 dead morning after exposure; 1/2 at 3rd day
520	268	2.21	0.79	3.2	2.1	Trace	Negative	0/10	1/2 at 17th day; 1/2 observed.
520	454	3.75	1.39	0.5	0.6	0.9	Negative	7/10	1/2 at 6th day; 1/2 at 8th day.
520	535	4.42	2.40	-	-		-	17/20 at 3rd day	1/2 at 3rd day; 1/2 observed.
1020	258	2.13	0.76	2.3	0.1	32.0	25	9/10	1/2 at 13th day; 1/2 observed.
1020	395	3.27	1.07	2.4	0.6	33.6	20	10/10 at 2nd day	1/2 at 7th day; 1/2 at 13th day.
1020	508	4.20	1.55	-	-	45.2		-	1/2 at 13th day; $1/2$ dead at 11th day.
1560	146	1.21	0.15	2.7	25.4	Trace	400	10/10 on withdrawal	1/2 dead within 24 hours.
1560	257*	2.13	0.13	25.2	60.8	1.1	500	10/10 on withdrawal	2/2 dead within 24 hours.

<sup>\*</sup> Exposure terminated after ca. 31/2 hrs due to plugging of combustion tube.

mortality (70-100%) resulted from products formed at lower temperatures but at higher concentrations (2.1 to 4.4 mg/liter).

No pathologic changes were seen in rats exposed to unheated XF-408 or the 520°-pyrolyzed fluid, when the concentration was 2.9 mg/liter or less. When the unheated fluid concentration was increased to 4.2 mg/liter, pulmonary hemorrhage and edema were found. Pulmonary edema was also seen in rats exposed to pyrolysis products formed at 520°, 1020°, and 1560° at concentrations of 3.8, 2.1, and 1.2 mg/liter, respectively.

Toxic signs in dogs exposed to unheated XF-408 were lacrimation, weight loss, conjunctivitis, salivation, cough, anorexia, depression, and dehydration. At the highest unheated concentration (4.2 mg/liter) ataxia and diarrhea were also noted. As the pyrolysis temperature was increased most of these signs persisted; in addition, respiratory distress, moist rales, and ocular and nasal discharge, all of which were most severe at 1560°, were observed. Both dogs of the group died within 24 hours after exposure to the 1560°-products at levels as low as 1.2 mg/liter.

Pathologic changes in dogs exposed to XF-408 aerosols varied. Time of sacrifice following exposure varied from a few hours to several weeks. The dogs held for periods of 14-17 days after exposure had partially recovered. Six of eight dogs exposed to various concentrations of unheated XF-408 showed varying degrees of acute tubular nephrosis. In addition, lung changes were

varied and consisted of moderate degeneration of bronchiolar epithelium with occasional small foci of bronchopneumonia. Changes produced by exposure to XF-408 pyrolyzed at 520° were essentially similar to those produced by the unheated aerosol. When the pyrolysis temperature was raised to 1020°F, the pathological picture differed considerably. No nephrosis was found, and lung changes were less severe. However, in one dog a severe case of bronchopneumonia was noted. From the clinical history of this animal it is believed this finding was more likely due to a pre-existing lung condition rather than to effects of the exposure. Three of four dogs exposed to the products from heating at 1560°F showed massive pulmonary edema, intrapulmonary hemorrhage, and destruction of bronchiolar epithelium. No changes were seen in the other organs examined.

The carbon monoxide content of the blood of two dogs exposed to 1.2 mg/liter of XF-408 heated to 1560°F, was 3.0 and 6.7 volume per cent. At a concentration of 2.1 mg/liter, two other dogs showed blood carbon monoxide contents of 4.5 and 3.2 volume per cent. The carbon monoxide concentration in the chamber was 400-500 ppm.

#### MLO-5277.

Chamber air analyses and mortalities in each species are shown in Table III.

Rats exposed to low concentrations of both

Table III

Toxicity to Rats and Dogs of Heated Aerosols of Hydraulic Fluid MLO-5277

Exposure period: 4 hours

	Fluid feed	Nominal	Char	mber ana	lyses	Mortality fraction	Time of sacrifice
Tube temp	rate	fluid	TPM	Alde- hydes	Carbon mono- xide	rats	dogs
°F	mg/min	mg/l	mg/l	ppm	ppm		
72	61	0.51	0.39	_	-	3/10	1/2 at 7th day; 1/2 at 14th day.
75	126	1.04	0.66	-		3/10	
73	153	1.26	0.76	0000	-	_	1/2 at 3rd day; 1/2 at 7th day.
73	248	2.05	1.10	-	-	-	1/2 at 1st day; 1/2 at 7th day.
71	339	2.80	1.48	-	-	-	2/2 at 3rd day.
520	60	0.50	0.45	_	-	1/10	1/2 at 1st day; 1/2 at 14th day.
520	117	0.94	0.68	-	-	5/10	C-matrices
520	242	2.00	1.61	-	-	-	2/2 at 11th day.
520	402	3.32	1.75	-	-		2/2 at 5th day.
520	480	3.98	3.02	11	-	7/10	1/2 at 1st day; 1/2 at 4th day.
1020	58	0.48	0.32	-	-	-	2/2 at 7th day.
1020	59	0.49	0.39	-	15	2/10	
1020	81	0.53	0.55	-	-	-	1/2 at 3rd day; 1/2 at 9th day.
1020	157	1.30	0.48	-		3/10	
1020	399	3.30	0.71	-	-		1/2 dead 36 hrs after exposure; 1/2 at 2nd day.
1020	468	3.88	0.72	16	50	10/10 at 2nd day	1/2 after withdrawal; 1/2 at 1st day.
1560	30	0.25	0.24	-	150	0/10	_
1560	78	0.64	0.26	-	150	3/10	1/2 at 1st day; 1/2 at 7th day.
1560	119	0.98	0.34	-	100	10/10	
1560	405	3.35	0.52	18	100	10/10 at 1st day	1/2 upon withdrawal; 1/2 dead during night follo ing exposure.

unheated and pyrolyzed MLO-5277 (0.3 to 0.6 mg/liter) showed the same toxic signs. These were a reddish secretion about the eyes and nose and respiratory distress appearing about one hour after exposure. At higher concentrations (0.9 to 1.3 mg/liter) the same signs were seen, but the respiratory distress was more marked and did not appear until the following day. At these levels mortality rates were essentially proportional to pyrolysis temperature, reaching 100% at 1560°F. At the highest concentrations (3.4 to 4.0 mg/liter) the reddish secretion was much more profuse, and severe respiratory distress was noted immediately after exposure. At these concentrations the mortality was very high (70 to 100%) from products formed at all pyrolysis temperatures.

Rats exposed to low concentrations of both pyrolyzed and unheated MLO-5277 (0.3 to 0.6 mg/liter) showed the following changes in the lung; slight congestion, alveolar collapse, and edema. Rats exposed to higher concentrations (0.9 to 1.3 mg/liter) showed more pronounced lesions; some rats exposed to the 1560°-pyrolysis products showed lung edema and hemorrhage. Rats exposed to still higher concentrations (3.4

to 4.0 mg/liter) of the pyrolyzed fluid showed extensive necrosis of the epithelium of the trachea and bronchial tree and massive pulmonary edema and hemorrhage.

Dogs exposed to low concentrations of both pyrolyzed and unheated MLO-5277 (0.5 to 0.6 mg/liter) showed at most some eye irritation. When the concentration of unheated fluid was increased (1.3 to 2.8 mg/liter), anorexia, depression, moist rales, rhinitis, and respiratory distress were noted. When the pyrolysis temperatures and the concentrations both were raised (520 to 1560° and 3.3 to 4.0 mg/liter), the severity of these signs increased and additional toxic signs, consisting of salivation, cyanosis, and ocular congestion, were noted.

Dogs exposed to low concentrations of unheated MLO-5277 (ca. 0.5 mg/liter) showed no pathologic changes when sacrificed up to seven days after exposure. Those exposed to similar concentrations of pyrolyzed fluid showed pulmonary edema and hemorrhage. Dogs exposed to higher concentrations of unheated fluid (1.3 to 2.8 mg/liter) showed pneumonia, lung edema, lung hemorrhage, and alveolar collapse; the severity of the lesions paralleled the concentration

of fluid. As the concentrations and the pyrolysis temperatures were increased, the pulmonary edema and hemorrhage increased correspondingly in severity. Dogs exposed to 4.0 mg/liter of 520°-pyrolyzed MLO-5277 or to 3.3–3.9 mg/liter of 1020° or 1560°-pyrolyzed fluid died rapidly. Pathologic examination showed severe necrosis of the epithelial lining of all airways with massive pulmonary edema and hemorrhage.

#### Discussion

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OS-40 caused severe toxic effects in rats and dogs only when they were exposed to the products of pyrolysis at the highest temperature (1560°) and at the highest concentrations (3.5 to 4.3 mg/liter). On the other hand, toxic effects of equal or greater severity were caused by lower concentrations and/or pyrolysis temperatures of the other two fluids. In this sense OS-40 might be considered to be the least toxic of the three fluids

The toxicity of the pyrolysis products of OS-40 may have been due to the formation of aldehydes, which was at a maximum at 1560°F. This was similar to the behavior of the ester-type lubricating oils previously investigated. Treon and others' showed that the toxicity of pyrolyzed phosphates commercial tricresvl depended largely upon the extent of decomposition of the molecule. This appears to be true for OS-40 also, since pyrolysis at 1560° produced the greatest quantity of decomposition products, (CO and aldehydes), and also the most severe toxic signs and pathology in exposed animals.

XF-408 was unique in this study in that it caused acute tubular nephrosis. This lesion was seen in dogs exposed to the unheated and 520°-pyrolyzed fluid, but not in animals exposed to the 1020°- or 1560°-pyrolyzed fluid. This finding suggests that the moiety in unheated XF-408 which caused this kidney lesion was not affected by heating at 520°F, but was destroyed at 1020° or higher temperatures. Pyrolysis of XF-408 at 1560° produced a fairly high concentration of inorganic fluoride: 61 ppm calculated as HF. However, this concentration of HF alone probably would not be capable of causing the high mortality and severe pathologic changes observed.<sup>14</sup>

When XF-408 was pyrolyzed at 1560° the chamber carbon monoxide concentration (400–500 ppm) and the maximum blood CO content of exposed dogs (6.7 volume per cent) were at levels which would ordinarily be considered non-toxic. However, Hays¹s has suggested that animals exposed to potent respiratory irritants for several hours may be extremely anoxic and that,

under such conditions, even relatively low levels of CO may no longer be regarded as non-toxic. Zapp¹o showed that the toxicity of carbon monoxide is greater in oxygen-deficient atmospheres than it is in air.

MLO-5277 may be considered the most toxic of the three fluids on the basis of the response of animals exposed to the unheated fluid and to low levels of the pyrolyzed fluid. Pyrolysis of MLO-5277 at 1020° and 1560° produced low concentrations of CO and aldehydes which could not account for the high mortality observed. The toxic degradation product was not identified.

Aside from the thermal stability of these fluids, their physical properties should receive some consideration. These fluids are insoluble in water and, when inhaled as aerosols in relatively high concentrations, may be retained by the lungs. During an extended exposure period sufficient amounts of these fluids may have accumulated in the lungs to account for the alveolar collapse, edema, and hemorrhage found in the exposed animals. During the short period of contact with the heated tube only a portion of the fluid may have been pyrolyzed, the amount depending largely upon the temperature. Consequently, the products inhaled probably consisted of an aerosolized mixture of decomposed and unchanged fluids as well as vapors of the decomposition products.

#### Summary and Conclusions

It was found that the aerosols (heated and non-heated) of the hydraulic fluids studied are eye and respiratory irritants. It was also observed that toxicity was generally proportional to the nominal concentration of the aerosol and to the temperature to which the aerosol was subjected, except in the case of dogs exposed to XF-408. Aerosols of this fluid (unheated and heated at 520°F) caused acute tubular nephrosis. This lesion was not observed in dogs exposed to the aerosol heated at 1020° or at 1560°F, although the incidence of deaths was greatest following exposure to the aerosol heated at 1560°F. OS-40 produced severe pathologic changes in rats and dogs only when the nominal concentration of the fluid heated to 1560°F was 3.5-4.3 mg/liter. The toxicity of OS-40 varied with the degree of decomposition of the heated aerosol and with the concentration of aldehydes and carbon monoxide formed as a result of the heating. Maximum effects were produced when the aerosol was heated to 1560°F

From the results of this study it was concluded that OS-40 is the least toxic of the three hydraulic fluids. MLO-5277 was considered to be

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the most toxic, since there were severe responses in animals exposed to unheated fluid and to low concentrations of the pyrolyzed fluid.

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# **Enhanced Toxicity of Ozone-Hydrogen Peroxide Mixtures**

JOSEPH L. SVIRBELY, Ph.D.,\* OLGA J. DOBROGORSKI, M.D.,\*\* and HERBERT E. STOKINGER, Ph.D.

Research and Technical Services Branch, Division of Occupational Health, U. S. Public Health Service, 1014 Broadway, Cincinnati 2, Ohio

Concern over enhanced toxicity of air pollutant mixtures has led to the experimental demonstration that hydrogen peroxide  $(H_2O_2)$  in concentrations of a few ppm killed animals after a brief exposure when inhaled with a nonlethal concentration (1 ppm) of ozone (O\_3). It was possible also to demonstrate a moderate degree of protection against otherwise lethal doses of  $H_2O_2$ , by a single prior exposure to  $H_2O_2$ . Moreover, cross tolerance against the organic peroxide, cumene hydroperoxide was afforded also by a prior exposure to  $H_2O_2$ . Particularly noteworthy, was a tolerance produced by a prior exposure to  $O_3$  to mixtures of  $H_2O_2$  and  $O_3$ , despite the fact that such mixtures normally show enhanced toxicity.

TOXICOLOGIC studies on air pollutants in this laboratory in the past have involved single substances. Emphasis has been centered particularly on ozone<sup>1, 2, 3</sup> because of its dominant role in oxidant smog and because of many conflicting viewpoints on its toxicity that have appeared in the literature.<sup>4</sup>

The toxicity of ozone per se has been established for small animals.5 It was further demonstrated that the observed toxicity could not be attributed to the presence of nitrogen oxides that may accompany ozone production.1 An extreme range of physiologic response, from the rapidly lethal effects of ozone under added stress of exercise,2 to the marked tolerance to acute effects developed from brief exposure to relatively low concentrations of ozone,1,5 were found in rats and mice. Experimental evidence in another laboratory has confirmed the above observations concerning ozone toxicity and tolerance.6, 7 Respiratory distress, generally noted in laboratory animals exposed to ozone, has also been reported in welders using the inert-gas-shielded are welding process<sup>8</sup> and in exposed human subjects.<sup>9, 10</sup>

Although considerable progress has been made concerning the toxicity of ozone alone, with regard to its lethal effects, tolerance and pulmonary irritation, reports of acute smog incidents have pointed to the distinct possibility that both direct and indirect effects of air pollutants on health may arise not only from single pollutants

but also from combinations as well. Accordingly a systematic study was initiated involving the interactions of common air pollutants, chiefly the important respiratory irritants, with ozone, in order to ascertain whether or not the degree of injury observed in exposed animals could be attributed to a possible potentiation of combinations of these irritants.

The present studies involved testing the injurious nature of the reaction of hydrogen peroxideozone mixtures. Kinetic studies in aqueous solutions have indicated that hydrogen peroxide may be associated with ozone and its reaction product with water and organic substances to generate free radicals, HO and HO2, as well as organic free radicals." Prior tests for possible ozone contaminants, such as oxides of nitrogen, hydrogen peroxide, and free radicals (HO2, OH, HO3, O4, etc.), in a specially constructed mass spectrometer failed to reveal significant amounts of these substances, however, but the possibility of free radicals and/or hydrogen peroxide could not be ruled out owing to the relative lack of sensitivity of the instrument.5 This investigation included therefore, (1) the determination of acute and subacute lethal effects of such mixtures, (2) the "spread" of tolerance, observable with ozone, to hydrogen peroxide, and (3) the cross tolerance to either component following brief exposures to nonlethal levels of hydrogen peroxide or ozone.

#### Methods

Rats (Wistar-derived), mice (Swiss-derived), and hamsters used in these experiments were

<sup>\*</sup> Present address: Robert A. Taft Sanitary Engineering Center, 4676 Columbia Parkway, Cincinnati 26, Ohio.

<sup>\*\*</sup> Present address: Department of Pathology, Cincinnati General Hospital, Cincinnati, Ohio.

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adult, male animals obtained from commercial sources and maintained in this laboratory for several weeks on a ration of Rockland Rat Diet prior to study. The basic diet and water were available to the animals at all times except when the exposures were in progress.

Exposures, with approximately one air change per minute, were routinely carried out in a glass chamber of 18.5 liters capacity, as previously described.<sup>5</sup> A large chamber of 100 ft<sup>3</sup> capacity, used exclusively for ozone inhalation studies,<sup>2</sup> was utilized on several occasions. The vapor air mixtures used in these studies were based on vol-

ume per cent.

Varying concentrations of ozone-air mixtures were prepared by diluting metered amounts of ozone, generated by passing three liters of air per minute through a plastic-dielectric ozonizer of a low-current-density type, with measured amounts of compressed air. The air used for the mixtures was scrubbed by passage through a fritted bubbler containing potassium dichromate in concentrated sulfuric acid, followed successively by columns of a mixture of soda lime plus silica gel and Drierite.4 Details concerning the construction and chemical analyses of the output of the ozonizer, as well as the absence of organic impurities in the scrubbed compressed air have been discussed in another report from this laboratory.5 Ozone determinations were made by the alkaline-potassium iodide method of Smith and Diamond12 as modified by Byers and Saltzman.13

The hydrogen peroxide-air mixtures were prepared by diluting the vapors, generated by the passage of either water-pumped nitrogen or scrubbed compressed air at specified rates through a bubbler containing approximately 12 ml of 90% H<sub>2</sub>O<sub>2</sub>, at room temperature, with scrubbed air, to give a final flow rate of about 20 liters per minute. This dilution rate was chosen because pilot studies, without animals, had indicated that assay values for the concentration of hydrogen peroxide in the chamber sampled at the outlet were about 50% lower than values at the inlet if one air change in four minutes was passed into the chamber in contrast to a difference around 8% with one air change in one or even two minutes. When animals were present in the chamber a similar discrepancy in analytic values at the sampling sites with one air change in four minutes had been noted with ozone.5 The rate of air flow for dilution undoubtedly plays an important role in the analyses of substances that are catalytically destroyed during passage through the chamber not only at the surface of the animals but also by absorption, adsorption, decomposition, condensation, or a

combination of these and other factors. Desired levels of hydrogen peroxide in the exposure chamber could not be obtained with Superoxyl (30% H<sub>2</sub>O<sub>2</sub>) even at elevated temperatures but were satisfactorily obtained with 90% H<sub>2</sub>O<sub>2</sub>. The measurement of concentrations of hydrogen peroxide in the air mixtures was based on the development of a stable yellow color with stock solution of titanium sulfate reagent. <sup>14, 15</sup> This reaction was found to be specific for hydrogen peroxide only since no color development was observed

with varying amounts of ozone.

Hydrogen peroxide-ozone mixtures were prepared by diluting metered amounts of these components, generated by methods outlined in preceding sections, with scrubbed air to a rate around 20 liters per minute. Concentrations of hydrogen peroxide in the mixtures were determined directly with the titanium sulfate reagent. As hydrogen peroxide was found to interfere with ozone analysis by its reaction via the alkalineiodide reagent, air samples of the mixtures were first passed through a bubbler containing a mixture of 4% CrO3 and 2.8% KMnO4 in distilled water (this scrubbing process removed the hydrogen peroxide but no ozone) and then into a second bubbler containing the alkaline-iodide reagent for ozone analysis. In order to allow for a correction blank for traces of oxidants from the first bubbler (liberating free iodine from the alkaline-iodide reagent), scrubbed compressed air was drawn into the two bubblers under similar sampling procedures employed for analyses of the mixtures. Pilot studies, without animals, indicated that satisfactory analytical determinations could be obtained for the components either individually or in combination. Analyses of these mixtures showed no rapid reaction between ozone and hydrogen peroxide in the vapor state, as previously stated (15).

Inhalation exposures of small laboratory animals to varying concentrations of the components of the mixtures, either singly or in combination, were generally limited to a single, four-hour period. Analyses of the inlet and outlet concentrations of vapor-air mixtures from the chamber were made at periodic intervals. Although differences of 10–20% between samples at the two sites previously were reported for ozone determinations, they were more pronounced with hydrogen peroxide, especially when larger animals (rats) were exposed. The discrepancey between analytic and calculated concentration for hydrogen peroxide has been discussed in a

report from another laboratory.14

Observations concerning the lethal effects of the mixtures in the animals were noted during and after exposure. Tissues from animals that died from the exposures or were killed subsequently with intraperitoneal injections of nembutal were prepared for routine histopathologie examination.

# Results of Exposures

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The mortality data observed among mice, rats, and hamsters after single, 4-hour exposures to hydrogen peroxide and ozone individually or in mixtures are presented in Tables I and II. Mice were found to be more sensitive test animals than rats for assaying the toxicity of a series of admixtures in which the ozone concentration was maintained around one ppm while the H<sub>2</sub>O<sub>2</sub> concentration for individual experiments was decreased successively from 16.6 to 1.5 ppm (Table I). Similarly in Table II, hamsters were shown to be more resistant to such mixtures, and also to higher levels of ozone than was indicated by previous reports.<sup>5</sup>

Examination of the data on the mortality in control groups shown in Tables III and IV yields greater information on the susceptibility of mice to single, 4-hour exposures to ozone or hydrogen peroxide alone. In such exposures, ozone becomes lethal to small rodents at 5–6 ppm. At 78 ppm  $H_2O_2$  alone is not lethal to mice on 4-hour expo-

Table I
Acute Inhalation Exposures (4 Hours) of Male
Rats and Mice to Hydrogen Peroxide-Ozone
Mixtures

chambe	rage r conc'n om)	Species	Average weight (gm)	Mortality following exposure  No. dead  No. exposed				
H <sub>2</sub> O <sub>2</sub>	Oa*		Avera (gm	24 hrs	2 weeks			
16.1		M	25	0/10	0/10			
		R	220	0/5	0/5			
	1.1	M	25	0/45	Used for other studies			
-	1.0	R	230	0/40	66 66 66 66			
16.0	0.90	M	27	3/10				
		R	252	1/5	1/5			
7.8	0.96	M	27	2/10	2/10			
		R	260	2/5	2/5			
3.3	1.0	M	25	3/10	3/10			
		R	272	0/5	0/5			
1.5	0.92	M	26	0/10	0/10			
		R	260	0/5	0/5			

M-Mice. R-Rate

sures; at 113 ppm, one of 10 mice died within 24 hours and 4 of 10 died within the two-week observation period (Table IV). At 194 and 226 ppm  $\rm H_2O_2$  the mortality among mice was somewhat greater. It is notable that deaths due to

Table II Species Variation of Rodents in Single Inhalation Exposures to Hydrogen Peroxide and Ozone

chambe	Average chamber conc'n (ppm)		ppm)		Mortality following exposure No. dead No. exposed			
H <sub>2</sub> O <sub>2</sub>	O3	Species	Average (gm)	Dura ext (ho	24 hrs	2 weeks		
24.8	1.5	H M	112 28	4	0/10 2/10	0/10* (Group A) 2/10		
35.7	7.6	H M	120 26	4 4	0/5 5/10	0/5* (Group B) 5/10		
	44.6*	Н	105	136	0/7	(Group C)		
_	16.9*	R	280	1		2 dead 10 min. before end of exposure 3 dead ½ hr after end of exposure 3 dead 1 hr after end of exposure 2 dead 1½ hrs after end of exposure		

H-Hamsters, R-Rats, M-Mice.

#### TABLE III

Responses of Mice Previously Exposed to Ozone When Challenged with Hydrogen Peroxide-Ozone Mixtures (4-Hour Exposures)

Ex- posure group	Chamber O <sub>3</sub> conc'n av. (ppm)	Re- covery period (days)	Challer dosages age cha conc (ppr	aver- mber 'n	ing ex No.	y follow- posure dead xposed
			H <sub>2</sub> O <sub>2</sub>	Oa	24 hrs	2 weeks
1	1.6	2	36.3		0/30	0/30
	Controls		37.4		0/10	0/10
2	1.6	3	_	5.7	0/30	0/30
	Controls		-	5.7	8/30	8/30
3	1.4	5-6	227.	-	7/25	14/25
	Controls		227.	-	5/25	22/25
4	1.6	4	34.5	5.6	0/30	0/30
	Controls		34.5	5.6	12/30	13/30

<sup>&</sup>lt;sup>6</sup> Exposure in large chamber, 100 cu. ft. capacity. The ozone used in this chamber was generated in a U. S. Air Ozonizer, Scottdale Ozone Company, Scottdale, Pa., as previously described.<sup>11</sup>

 $<sup>^{\</sup>circ}$  Large chamber, 100 cu. ft. capacity used for ozone exposure. Group A and B challenged about 6 weeks after end of observation period for 70 minutes with 38 ppm  $O_4$ .

<sup>1/10</sup> and 3/10 hamsters in Group A dead 3 and 6 hrs. respectively after end of exposure. Surviving hamsters in Groups A, B, C, sacrificed 29 hours, 76 hours, and 6 days after end of exposure for pathologic observation.

TABLE IV

Responses of Mice Previously Exposed to Hydrogen Peroxide when Challenged with Hydrogen Peroxide-Ozone Mixtures (4-Hour Exposures)

H <sub>2</sub> O <sub>2</sub> chamber concentration (ppm)	Re- covery period (days)	Challer dosages age cha conc (ppr	aver- mber 'n	No.	y follow posure dead xposed
		H <sub>2</sub> O <sub>2</sub>	Oz	24 hrs	2 weeks
5.6 (5 days)†	3	_	5.4	2/10	3/10
Controls		-	5.4	6/10	7/10
5.6 (5 days)†	3	78.1	-	0/10	0/10
Controls		78.1	-	0/10	0/10
5.6	4	-	5.6	4/10	4/10
Controls		-	5.6	7/10	7/10
16.3	2	-	5.8	9/10	10/10
Controls		-	5.8	9/10	10/10
46.2	3	-	5.8	1/10	1/10
41.4	2		5.6	3/10	4/10
Controls		-	5.6	8/10	8/10
40	7	113.	-	0/10	2/10
Controls		113.	-	1/10	4/10
40	8	194.	-	0/10	2/10
Controls		194.	-	3/10	6/10
44	6	-	6.1	7/10	7/10
Controls		-	6.1	10/10	10/10
44	4	226.	-	1/10	5/10
*C.H.(1/5 LC 50)		226.	-	1/10	1/10
Controls		226.		1/10	5/10

\*C. H. (Cumene Hydroperoxide)—6-hour exposure to 100 ppm (inlet calculated concentration).

 $\dagger$  Exposure 4 hours per day for 5 days at 5.6 ppm  $\rm H_2O_2$  .

 $\mathrm{H}_2\mathrm{O}_2$  were in general, more delayed than was the case with lethal exposures to ozone.

Respiratory distress, indicated by spasmodic breathing and characteristic of ozone intoxication, was observed in a majority of the animals exposed to mixtures of hydrogen peroxide-ozone. Recovery from the respiratory distress generally occurred in hamsters within 24 hours following a 4-hour exposure to 9–12 ppm O<sub>3</sub>; however, hamsters exposed to 40 ppm O<sub>3</sub> for about one hour still exhibited labored breathing 72 hours following the end of exposure.

Histopathologic examination of organs of sacrificed mice and rats exposed to lethal or near-lethal concentrations of ozone showed intense pulmonary edema, congestion of interstitial areas, massive extravasation of red blood cells into alveoli and mobilization of macrophages. Other changes included recent congestion in the liver and kidney as well as a depletion of lipoid

from adrenal cortices. It was not possible by histopathologic examination to differentiate between alterations in tissues characteristic of ozone itself, and possible ozone contaminants such as oxides of nitrogen, free radicals or other oxidants.

In other inhalation studies, edema, desquamation of tracheal and bronchial epithelium, engorgement of lung parenchyma, a moderate degree of engorgement of the liver and depletion of cortical lipids were generally observed in rate and mice exposed to mixtures of hydrogen peroxide (13–25 ppm) and ozone (1.2–2.9 ppm). The extent of the pathologic changes depended on duration of survival. Exposure to these mixtures appeared to produce histologically visible edema sooner than from ozone alone.

Histopathologic examination of various organs of hamsters exposed to 44.6 ppm of ozone (Table II), indicated that primarily the lungs were affected. Edema was noted 29 hours post exposure; the fluid was stringy and apparently of a low protein content, and partial desquamation of the bronchial epithelium was present. Observations 76 hours post exposure included absence of edema, regeneration of bronchial epithelium, thickening and infiltration with chronic inflammatory cells of the ductal, as well as adjacent alveolar walls, a marked hyperplasia of the alveolar lining cells, marked dilation of alveolar ducts and periplural alveoli.

The histologic picture, 6 days post exposure, showed disappearance of acute dilatation of the ducts and regression in the peribronchiolar and periductal monocytic infiltration. The bronchiolar and ductal walls were slightly puckered and tortuous, and resembled those found in long-term ozone exposures<sup>3</sup> except for lack of fibrosis.

#### **Cross Tolerance**

Previous studies in this laboratory<sup>2, 5</sup> have demonstrated that a marked tolerance to normally acute lethal exposures to ozone develops in rodents following initial exposures to relatively low ozone concentrations. This is illustrated by exposure group 2 in Table III where all the preexposed mice survived a subsequent challenge dose of ozone which killed 8 of 30 control animals receiving no preexposure. Respiratory distress, characteristic of ozone exposures, was still observed in the preexposed animals during and following the challenge dose.

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The data in Table III indicate that a tolerance similar to that found for ozone may also be established against lethal levels of H<sub>2</sub>O<sub>2</sub> alone (Group 3) or against mixtures of H<sub>2</sub>O<sub>2</sub>-O<sub>3</sub> (Group 4). This capacity of O<sub>3</sub> to cross-protect

animals against subsequent exposures to  $H_2O_2$  is remarkable, but is all the more remarkable in protecting animals against a mixture of  $H_2O_2$ - $O_3$  which in itself is a highly potentiating toxic mixture as shown in Table I. Other responses to exposure, however, such as hyper-excitability, eye irritation, emaciation and delayed deaths, generally observed in mice exposed to very high levels of  $H_3O_2$ , still occurred and were not suppressed by the tolerance following a challenging dose of the components, either singly or in admixture.

## Preexposure to H<sub>2</sub>O<sub>2</sub>

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Table IV presents mortality data on groups of mice preexposed to  $\rm H_2O_2$  and subsequently challenged by normally lethal exposures to either ozone or hydrogen peroxide. Five days exposure at 5.6 ppm  $\rm H_2O_2$  and single exposures to 40 ppm  $\rm H_2O_2$  resulted in marked tolerance to subsequent challenge doses of ozone. A single exposure to 5.6 ppm  $\rm H_2O_2$  conferred somewhat less tolerance; a single exposure to 16 ppm  $\rm H_2O_2$  resulted in no tolerance. This apparent anomaly is probably related to the difference in recovery period after preexposure in these two experiments.

A single preexposure at about 40 ppm  $H_2O_2$  yielded a certain, perhaps equivocal, degree of tolerance to subsequent challenge doses of  $H_2O_2$  (113-226 ppm).

A comparable preexposure to an organic peroxide, cumene hydroperoxide, at one-fifth of its  $LC_{\infty}$  provided a measure of tolerance to lethal concentrations of  $H_2O_2$ .

Histopathologic examination of the lungs of mice exposed during these studies and killed 7 to 16 days after the last exposure were similar to those reported for mixtures of  $H_2O_2$  and  $O_3$ .

### Preexposure to H<sub>2</sub>O<sub>2</sub>-O<sub>3</sub> Mixture

Two groups of hamsters were exposed to mixtures of  $\rm H_2O_2$  and  $\rm O_3$  in the experiments reported in Table II (Group A and Group B). All these hamsters survived these exposures and subsequently were challenged 7 to 8 weeks later with 38 ppm  $\rm O_2$ .

Group A, preexposed to the mixture at lower concentrations, showed a moderate amount of edema six hours after the challenge exposure. At 29 hours post challenge, findings were similar to those previously described for hamsters exposed to ozone. A very pronounced cellular reaction in peribronchial and periductal tissue was evident in hamsters sacrificed 76 hours post challenge.

The higher concentration of preexposure mixture was given Group B. At 29 hours post

challenge there was no edema, but evidence of peribronchiolar reaction was seen. The cellular reaction (76 hours post challenge) of peribronchial and periductal tissues was comparable to that observed in the above group.

These findings in hamsters again stress that two separate sites of action, or possibly two separate mechanisms of tissue damage may be involved in ozone intoxication. Although preexposure to ozone may decrease the amount of edema in the lung from a challenge dose, only slight reduction in cellular reaction was noted in the bronchiolar and ductal tissues.

#### Comment

Observations, based on mortality and histopathologic examination of lungs of mice, a species found to be slightly more sensitive than rats, but far more susceptible than hamsters in these inhalation studies, suggest that the toxicity of mixtures of hydrogen peroxide-ozone was more pronounced than that of either component separately. Hydrogen peroxide in concentrations 3.3 ppm and above enhanced the toxic effects of levels of ozone (around one ppm) normally not acutely injurious in mice; concentrations of 7.8 ppm H<sub>2</sub>O<sub>2</sub> and above increased the toxicity of ozone in rats. Pulmonary edema, characteristic of moderately high levels greater than 2 ppm ozone was not found in mice exposed to levels of H<sub>2</sub>O<sub>2</sub> around 226 ppm, but some hyperplasia of the bronchial epithelium was observed 12 days later. Exposures to mixtures of H<sub>2</sub>O<sub>2</sub> (13–25 ppm) and ozone (1.2-2.9 ppm) appeared to accelerate the production of histologically visible edema and periductal cellular reaction more than that seen from exposure to ozone alone.

Histopathologic examination of the lungs of ozone-exposed animals indicated that two sites or mechanisms of tissue damage appear to be involved in the response, whereby a marked tolerance to acute lethal effects of ozone developed in rodents following initial exposure to relatively low ozone concentrations. Although pre-exposure to ozone decreased the amount of pulmonary edema following a challenging dose, little reduction in the cellular reaction of peribronchial and periductal tissue was noted. Moreover, respiratory distress, indicated by labored breathing and characteristic of ozone intoxication, was still observed in the "tolerant" animals. It should be emphasized here that this tolerance does not prevent the development of fibrotic changes in the lungs produced from daily repeated exposures to low levels of ozone.3 We have no explanation for the mechanism of the enhanced toxicity of the H<sub>2</sub>O<sub>2</sub>-O<sub>3</sub> mixture.

A tolerance comparable to that found for ozone was generally observed in mice challenged with high doses (above 113 ppm) of H<sub>2</sub>O<sub>2</sub> following pre-exposure around 1/5 LD50 level of H2O2 (40 ppm), H<sub>2</sub>O<sub>2</sub> provided also a cross-tolerance to O<sub>3</sub> in mice, and to an organic peroxide, cumene hydroperoxide. This cross tolerance afforded by substances possessing a common chemical grouping, namely peroxide, suggests a common mechanism of action. Because of the well known capacity of peroxides to denature protein, an immuno-chemical mechanism may account for at least part of the observed tolerance; there is some indication from biochemical studies that adaptive enzymes may be involved. Substances such as serotonin, adrenalin or histamine or other factors may also be involved. The "spread" of tolerance to other pulmonary irritants should be studied further.

The question of whether possible ozone contaminants, such as free radicals or other oxidants, affect the toxicity of ozone in laboratory animals, will have to be investigated by more sensitive analytic procedures, and histochemical or enzymatic tests, since histopathologic examination failed to resolve the issue.

#### Summary

1. Hydrogen peroxide in concentrations of 3.3 ppm and above enhanced the toxic effects of usually noninjurious doses of ozone (around one ppm) in mice; concentrations of 7.8 ppm and above of H<sub>2</sub>O<sub>2</sub> were required in rats.

2. A moderate degree of protection to lethal challenging doses of hydrogen peroxide, comparable to the tolerance developed by ozone for itself, developed after initial exposure of mice around 0.2 LD<sub>20</sub> level of H<sub>2</sub>O<sub>2</sub>.

3. A cross-tolerance to the acute lethal effects of ozone can likewise be developed in mice after repeated exposure to low levels of H<sub>2</sub>O<sub>2</sub> or a single dose about 0.2 of the lethal dose. An initial exposure to approximately 0.2 LD<sub>20</sub> of H<sub>2</sub>O<sub>2</sub>, or to an organic peroxide, cumene hydroperoxide, also afforded tolerance to a challenging dose of hydrogen peroxide. A tolerance to ozone was developed in hamsters, although this species is more resistant to the toxic effects of ozone than mice and rats. The development of tolerance to

a mixture of H<sub>2</sub>O<sub>2</sub> and O<sub>3</sub> with potentiated toxicity, from a prior exposure to ozone seems particularly noteworthy.

# Acknowledgment

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# The Influence of Radioactivity and Lung Burden on the Pulmonary Clearance Rate of Barium Sulfate

H. CEMBER, Ph.D.,\* J. A. WATSON, M.S., and M. E. NOVAK, B.S.†

Department of Occupational Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

Parallel groups of rats were injected intratracheally with radioactive and non-radioactive barium sulfate particles which had a geometric mean size of 3.6 microns. Dose levels were 23.3, 233, and 2330 micrograms. Lung clearance was found to be exponential in time. All doses had a clearance half-time of about 10 days, except the 2330  $\mu$ g radioactive dose for which the half-time was 27.5 days.

#### Introduction

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STUDIES in this laboratory on the carcinogenic effects of radioactive dusts strongly suggested that pulmonary retention is one of the main factors in determining the hazard from inhalation of such dusts. Data on radioactive barium sulfate1 and radioactive cerium fluoride2 showed that a long continuing radiological insult to the lungs is more likely to result in bronchogenic carcinoma than a more intense insult in which the same total amount of energy is absorbed over a shorter period of time. To accurately assess the carcinogenic hazard of a radioactive atmosphere, therefore, the quantitative relationship among absorbed dose rate, total absorbed dose, and probability of carcinoma must be known. Absorbed dose rate to the lung, of course, is dependent on the pulmonary clearance rate. The lung appears to behave like a two or three compartment reservoir, each compartment having its own characteristic clearance rate. According to this model, the compartment that is cleared most rapidly is thought to represent the upper respiratory tract, the next one to be the deep respiratory tract and the third one, when a third compartment is found, may be the one in which the particulate matter is bound to the lung tissue. A good example of such a three compartment system is reported by Albert and Arnett.3 In observations taken on humans who inhaled isotopically labelled iron dust, they

found one clearance period of about two to four hours, in which about 47% of the dust was cleared, a second period of 31 to 36 hours in which another 21% of the lung burden was cleared, and a third period in which there was little or no clearance over a 70-day observation period. Great differences in clearance half times from the deep respiratory tract have been reported. In this laboratory, for example, a study of the clearance of 1.45 micron BaSO, particles revealed a deep respiratory tract half-time of two days, while Hodge found a 60-day half-time for UO2 particulates.4, 5 Even longer residence time has been reported in semi-quantitative terms. Dutra, for example, in studying beryllium oxide particulates, found that "large amounts of dust remained within the lungs of rats for over a year after the last period of exposure, and there was little tendency for the beryllium to be redistributed from the lungs to other tissues."6 It is precisely this great range in deep pulmonary tract retention times that makes the estimation of the radiological risk from a radioactive atmosphere so difficult.

Although the exact reasons for the observed differences in lung clearance rates are not well known, clearance rates are thought to be influenced by certain factors, such as particle size distribution and magnitude of the lung burden. With respect to the influence of particle size on lung clearance, for example, Albert and Arnett found that iron particles whose mass median diameter was 3.7 microns were eliminated much more rapidly than 1.9 micron particles. About 92% of the larger particles were cleared in nine hours, while only 68% of the smaller particles left the lung after 36 hours. A major part of this difference, of course, is due to the fact that a

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<sup>\*</sup> Present address: University of Cincinnati, College of

Medicine, Cincinnati 19, Ohio.
† Present Address: University of Florida, Gainesville,

greater proportion of the larger particles were deposited on the ciliated epithelium, and hence were readily transported out of the lung. That fraction deposited in the deep respiratory tract, however, had a much larger retention time in the case of the smaller particles, 33 hours in comparison to three to six hours. The work of Stokinger et al. is an example of a similar finding in the case of rats who inhaled dusts of uranium compounds.7 These investigators found that 44% of U<sub>3</sub>O<sub>8</sub> particles whose mass median diameter was 2.6 microns were cleared out of the lung in slightly less time than 23% of the lung burden of 0.45 micron UO2 particles. It is interesting to examine some of these data from another point of view. In the experiments cited above, the dust retained in the lung was measured in terms of mass, or mass equivalent. If the mass is converted to number of particles, however, it is immediately apparent that more small particles are moved per unit time than large particles. In the case of the uranium dusts, for example, 44% of the lung burden of 2.6 micron particles corresponds to 1.3 × 10° particles, while 23% of the 0.45 micron dust includes 2 × 108 particles.

The distinction between mass removal and individual particle removal is important in the ultimate understanding of the mechanism of pulmonary clearance. This distinction is especially important in view of LaBelle and Brieger's finding that the rate of clearance increases with the magnitude of the lung burden.5 They reported a correlation among lung burden, number of phagocytes within the lung, and clearance rate. Their data suggest that the release of phagocytes into the lung is stimulated by the dust in the lung, and that pulmonary clearance rate goes up after the phagocytic mechanism is triggered. The effect of lung burden on the pulmonary mechanism was tested in the experiment described below. In view of the possible radiation effects on the pulmonary clearance mechanism, and because of Heller's finding that radioactive particulates are phagocytosed in vitro at a slower rate than their non-radioactive counterparts, the clearance of radioactive particles was studied in the same experiment. Groups of rats were exposed to radioactive and non-radioactive barium sulfate particulates at three different lung burdens. The rats were serially sacrificed over a 21day period, and the quantity of barium sulfate remaining was determined. From these data, all of which fell on exponential curves, the retention half-times were calculated.

#### **Experimental Technics**

Six groups of female albino rats of the National Laboratory's Wistar strain, whose weight distribution was 393 ± 34 grams, were intratracheally injected with barium sulfate particulates. The treatments included BaS32O4 and BaS<sup>35</sup>O<sub>4</sub> at levels of 23.3, 233, and 2330 micrograms per rat; each group received a different one of the six possible doses. All the particles were labelled with 0.19 microcuries gamma emitting Ba133 as a tracer, thereby permitting radiometric determinations with a gamma ray spectrometer. One millicurie of S35 was included in every dose of the radioactive particles. The initial radiation dose rate from the S35 was 70 rad per hour, while the initial radiation dose rate from the Ba133 was 0.013 rad per hour. Both the radiobarium and radiosulfur were purchased from the Oak Ridge National Laboratory, the barium as BaCl2 in 1N HCl at a specific activity greater than 5 millicuries per gram barium and the sulfur as carrier free H2SO4 in 0.1N HCl.

The particulates were prepared by precipitation following the addition of BaCl<sub>2</sub> to Na<sub>2</sub>SO<sub>4</sub> solutions. In the case of the BaSa5O4 particulates, the H<sub>2</sub>S<sup>35</sup>O<sub>4</sub> was isotopically adulterated with non-radioactive H2SO4 to achieve specific activities of 313, 31.3 and 3.13 curies per gram sulfur, corresponding to one millicurie for each treatment level. The barium was labelled by mixing inert with radioactive BaCl2; the total Ba133 activity injected into each rat was 0.19 microcuries. After isotopic adulteration, both solutions were neutralized with NaOH, and the BaCl, was diluted to 0.001N while the Na2SO4 was diluted to 0.002N. After precipitation, the particles were washed three times in distilled water, and were resuspended in isotonic saline at concentrations of 1.553, 15.53, and 155.3 milligrams per milliliter. This treatment resulted in a particle size distribution of 3.6 × 1.05 microns.

Exposure was effected by intratracheally injecting 0.15 milliliter of the suspension into the lung. To inject the BaSO, into the lungs, the rat was anaesthetized with pentobarbital (Nembutal) and was placed on a special fixture which afforded easy access to the trachea through the mouth. A blunt 18 gauge by two inches long hypodermic needle was used to cannulate the trachea, and the injection was made with a 2-inch long 22 gauge needle that reached to the end of the cannula. After injection, 10 milliliters air was blown through the cannula into the lungs. Autoradiographs of lung sections showed fairly good intrapulmonary distributions of par-

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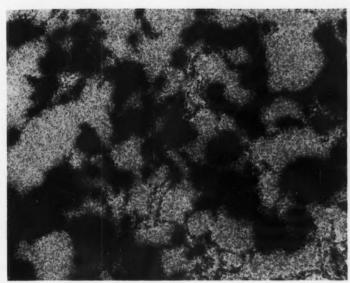


Figure 1. Autoradiograph of lung of a rat sacrified one day after injection of radioactive barium sulfate.

ticulates. Deposition of particles in the deep respiratory tract is illustrated in Figure 1, an autoradiograph made from the lung of a rat sacrificed one day after injection.

After treatment, the rats were caged in groups of four, and permitted to eat and drink freely until sacrifice. Periodically, during a 20-day period, groups of four rats each were sacrificed, and their lungs, livers, kidneys, spleens, femurs, and tracheobronchial lymph nodes were excised, digested with nitric acid, and radiometrically analyzed for BaSO4. Two of the rats in the group that received 2330 micrograms BaS<sup>22</sup>O<sub>4</sub> were kept in metabolism cages for 21 days, and the urine and feces were separately analyzed for radiobarium. To determine whether or not the barium activity in the lung was due to BaSO. or to dissolved barium that had been bound by the lung tissue, a group of rats was intratracheally injected with BaCl<sub>2</sub> solution, sacrificed over an interval of three minutes to three hours after injection, and had the lungs, blood, and other organs analyzed for radioactivity. It was found that the barium passed very rapidly out of the lungs into the blood stream. More than 99% of the barium was out of the lung in three hours, thereby showing that BaSO, which had undergone solution in the lung introduced very little error in the determination of the BaSO4 actually residing there.

#### Results

Pulmonary clearance in all cases was found to follow first order kinetics. The clearance rate, or slope of the pulmonary retention curve, was determined by a least square fit of the data. In the case of the group of rats that had received 2330 micrograms BaS<sup>22</sup>O<sub>4</sub>, for example, the equation for the deep respiratory retention curve shown in Figure 2 is

$$I = 1770 e^{-0.078t}$$

where I is the number of micrograms BaSO<sub>4</sub> in the deep respiratory tract t days after exposure. The half retention time corresponding to a clearance rate of 7.8 per cent per day is nine days. The retention half-times for all six treatment levels are given in the table below:

Talasted Dans	Half-time, Days					
Injected Dose, µgms.	BaS <sup>32</sup> O <sub>4</sub>	BaS35O4				
23.3	8 ± 11/2	13½ ± 1½				
233	10 ± 1	11 ± 1				
2330	9 ± 1	271/4 ± 41/2				

That the BaSO<sub>4</sub> eliminated from the lung passed out of the body is shown by the data

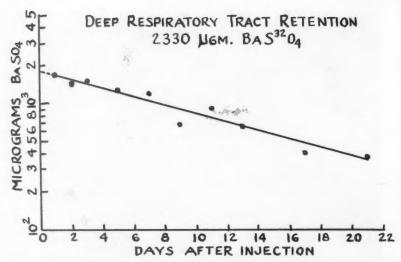


FIGURE 2. Graph of the retention time for barium sulfate in the deep respiratory tract.

from the other organs and from the excreta. Extremely small quantities of barium, less than 0.1 per cent of the injected dose, were found in all the organs that were analyzed. It is especially noteworthy that the tracheobronchial lymph nodes had very little radiobarium, and that the activity present in these lymph nodes varied in an unsystematic manner. The main avenue of elimination was the gastrointestinal tract; 1334 micrograms BaSO, (or the equivalent amount of barium) was collected in the feces during the first 10 days after exposure, and only 118 micrograms BaSO, was recovered from the urine during the same time period. The cumulative values for the feces and urine during the 21-day observation period was 1636 and 178 micrograms BaSO, respectively, or a total of 1814 micrograms. The quantity collected in the excreta of the rats confirms the observed 9-day half life in the deep respiratory tract. The extrapolated value at zero time of the deep respiratory tract retention curve, 1770 micrograms, represents the quantity of BaSO, actually deposited in the deep lung. The particulate matter in the upper respiratory tract is thought to be cleared within one or two days. After 10 days, therefore, and if there is no significant uptake of barium by any organ, the excreta may be expected to contain about 2330 - 1770 +  $(\frac{1}{2} \times 1770) = 1445$  micrograms BaSO<sub>4</sub>. The quantity actually collected was 1452 micrograms. This degree of agreement is remarkably good. For the entire 21-day period, if clearance from the lung had continued at the same rate, about 1888 micrograms BaSO<sub>4</sub> should have appeared in the excreta. The observed quantity was 1814 micrograms, again in excellent agreement with the pulmonary clearance data. The accumulation of tagged barium in the excreta is shown graphically in Figure 3. The data for the cumulative BaSO<sub>4</sub> in the feces were fitted by the two component equation

$$F = 800 (1 - e^{-0.56t}) + 1300 (1 - e^{-.058t}),$$

where F is the total number of micrograms  $BaSO_4$  collected in the feces during t days after injection. The short lived component probably represents the accumulation of  $BaSO_4$  particulates that were eliminated from the upper respiratory tract by ciliary activity and swallowed. The long lived component may represent particles which had been cleared from the deep respiratory tract, presumably by phagocytes which, after engulfing the particles, then migrated to the ciliated epithelium and were subsequently transported by the ciliary escalator to the mouth.

The fact that a significant amount of barium was collected in the urine clearly shows the relatively insoluble BaSO<sub>4</sub> to be soluble under the physiological conditions existing either in the lung or in the stomach, or in both organs. The urinary accumulation of barium is described by the simple exponential

$$U = 230 (1 - e^{-.069t}).$$

In this equation U is the number of micrograms

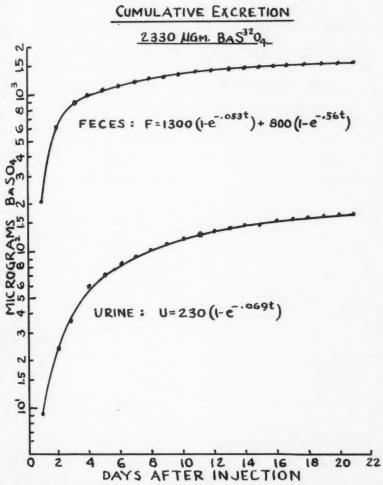


FIGURE 3. Graph showing the accumulated radioactive barium sulfate excreted in the urine and in the feces over a period of 21 days following injection.

of BaSO<sub>4</sub> corresponding to the observed barium content of the urine.

#### Discussion

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The results of this experiment show the clearance of BaSO<sub>4</sub> particles from the lung to be independent of the lung burden over the range of 23.3 to 2330 micrograms. It should be noted that the retention half time observed in this experiment was considerably greater than that reported in the experiment using 1.45 micron BaSO<sub>4</sub> particles. The reason for this discrepancy is not immediately apparent since the only dif-

ference between the two experiments was in particle size, and the observed discrepancy is in the opposite direction from that which would be predicted on the basis of particle size alone.

If the main mechanism of dust removal from the deep respiratory tract is phagocytosis, then the data from this experiment are compatible with the hypothesis that increased lung burdens of dust stimulate the production of alveolar phagocytes. If this is indeed true, then the data suggest that the increase in number of phagocytes is proportional to the increase in lung burden, and therefore there is no net effect on the clearance rate. The data are also amenable to explanation by Gross's theory regarding alveolar clearance. According to this theory, particles are propelled upward towards the ciliated epithelium by virtue of the fact that a viscosity gradient exists across the fluid film covering the alveolar surface, and the pulmonary excursions provide the motive force for this clearance mechanism. Any reduction in the excursions of the respiratory surfaces should therefore result in alveolar stasis and consequently longer retention time for dusts. Clearance rates, therefore, for any particular dust, should not be strongly dependent on lung burden provided the dust does not alter the respiratory pattern.

The results of this experiment also show that an interaction exists between lung burden and radioactivity level of the dust that inhibits the pulmonary clearance mechanism. This interaction, however, is observable only at relatively high lung burdens. This effect is surprising, since the same amount of radioactivity, one millicurie S35, was deposited in all cases, and consequently the specific activity of the particles varied inversely with the lung burden. The interaction between lung burden and activity level, if it is found to exist for other particles too, is of great significance in assessing the lung hazard from inhaled radioactive dusts. Experimental work already completed, suggests that continuing low level radiological insult to the lungs may lead to bronchogenic carcinoma. This means that for a given total absorbed dose, a dust of long pulmonary retention time is more hazardous than another dust of shorter pulmonary retention time. Since a point may be reached at which the lung burden reduces the clearance rate of radioactive dust, the implication of this experiment is that for a given amount of inhaled particulate radioactivity, the lung hazard may be an inverse function of the specific activity of the dust particles.

This is indeed an unexpected observation, and, because of its profound significance, great care must be exercised in the interpretation of the experimental data. The discrepancy between the clearance half-time observed in this experiment and that observed with smaller BaSO, particulates has already been noted. This unexplained difference, a factor of 5 (2 days vs. 10 days), is greater than the statistically significant difference between the 2330 micrograms BaS35O4 treatment and all the other treatments (10 days vs. 27 days) that was observed in this experiment. The discrepancy discussed above may be due to the fact that intratracheal injection, despite the fact that it is an extremely useful technic for the deposition of particulate matter in the lung

for the purpose of toxicological studies, is, because of the non-physiologic method of deposition, not a suitable exposure technic for studying lung clearance. Nevertheless, because of the importance of the possible inferences to be drawn from this experiment, more experimental data must be obtained, preferably by inhalation, in order to evaluate the validity of the inference drawn from this experiment; that for a given total absorbed dose from inhaled radioactive dust, the radiologic risk may be dependent on the total amount of inhaled dust (or on specific activity of the particulates) and furthermore, that the direction of increasing risk is toward lower specific activity of the inhaled particulates.

### Summary

Barium sulfate particles, whose mean size was 3.6 microns, were intratracheally injected into three groups of rats at dose levels of 23.3, 233, and 2330 micrograms; three parallel groups of rats were similarly treated with BaS<sup>35</sup>O<sub>4</sub> that contained one millicurie S<sup>35</sup> per dose. The rats were serially sacrificed over a 21-day period, and the barium sulfate left in the lung was determined. In all cases, the lung clearance was found to be exponential in time. The 2330 microgram dose of BaS<sup>35</sup>O<sub>4</sub> had a deep respiratory tract retention half-time of 27½ days, while all the other doses, both radioactive and non-radioactive, had clearance half-times of about 10 days.

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# Exposure of Mice to Nitrogen Dioxide a Constant Pressure System

ERIC G. COMSTOCK, M.D.\*, and ROGER R. RUE

Department of Biochemistry, Baylor University College of Medicine, Houston, Texas, and
Pulmonary Function Laboratory, United States Veterans
Administration Hospital, Houston, Texas

#### Introduction

Investigation of the respiratory effects of NO<sub>2</sub> requires a method for delivering a constant known concentration of NO<sub>2</sub> into an animal chamber. Continuous delivery of low concentrations of NO<sub>2</sub> cannot be accomplished with commonly available gas flow meters. Ripperton and Johnston¹ obtained dilute concentrations of NO<sub>2</sub> by displacing with air an intermediate concentration of NO<sub>2</sub> from a series of bottles into a main flow of air entering an animal exposure chamber. The concentration of NO<sub>2</sub> in the chamber varied from 0.7 ppm immediately after charging the bottles with NO<sub>2</sub> to 0.15 ppm after 23 hours. The present paper presents a modified system for maintaining a more nearly constant concentration of NO<sub>2</sub>.

# Materials and Methods

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The system utilizes a plastic bag made of 6 mil polyethylene which is available from most large building supply companies. It is 23 by 24 inches and made by folding a sheet of polyethylene and sealing the edges under waxed paper with a household iron. We have demonstrated that no measureable amount of 100% NO<sub>2</sub> will diffuse through a 12 square inch piece of this polyethylene material during a 24 hour period. Condensation of water within the plastic bag when not in use is controlled by adding a small amount of indicating silica gel (baker analized reagent). Before the bag is used, the silica gel is shaken out and contained air is removed by evacuation with a water aspirator.

Bottles A and B are 5 gal distilled water bottles which have been calibrated in liters.

The mouse exposure chamber is a one liter low form culture flask with the neck cut off to fit a large rubber stopper. This glass chamber allows continuous observation of the mice and eliminates excessive dead space.

Sampling and injection sites consist of a glass tube with a short side arm capped with a sleeve-type serum bottle stopper.

Indicating silica gel (Baker analized reagent) is used to dry the air before it is mixed with NO<sub>2</sub>. The NO<sub>2</sub> was obtained in lecture bottles from Matheson, Coleman and Bell.

# Operation of Exposure System

Figure 1 is a diagram of the system designed for exposure of mice to various concentrations of NO2 at a flow rate of approximately 500 ml of gas per minute for a total of 30 minutes. To charge the system bottle A is filled with the diluent gas, in this case with room air or oxygen, and allowed to come to atmospheric pressure. Valve V1 is positioned to allow tap water to enter bottle A. Valve V2 is positioned to allow gas forced from bottle A to flow through the desiccator trap. Valve V<sub>3</sub> is positioned to allow dry air to enter the previously evacuated polyethylene bag which is suspended freely by the top edge and may be supported partially from below. To avoid significant positive pressure in the bag the total charge of gas never should exceed about 30% of the volume of the bag.

To obtain a calculated volume of NO2 at atmospheric pressure a three foot section of plastic tubing is attached to a lecture bottle containing pure NO2. The valve on the bottle is opened to allow the gas to flow through the tube slowly against atmospheric pressure. After the tube has been flushed with gas which is released into a well ventilated hood, a 20 gage needle on an oiled syringe is passed through the wall of the plastic tubing and the required amount of NO2 is withdrawn. To allow for equilibration of pressure the syringe is left in place for one minute. The NO2 is injected into the flow of dry air as it enters the plastic bag. The syringe is flushed several times with dry air before it is removed from the line. After a measured quantity of gas

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<sup>\*</sup> This investigation was carried out during the tenure of a Postdoctoral Fellowship from the Division of General Medical Sciences, United States Public Health Service.

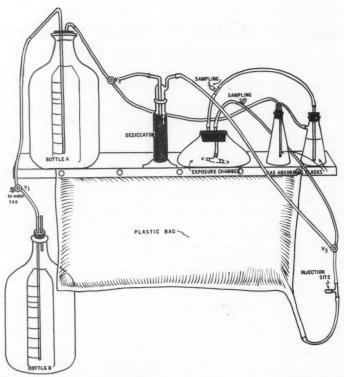


FIGURE 1. Diagram of an apparatus for exposure of mice to low concentrations of  $NO_2$ . Valve  $V_1$  is connected to a water supply and a measured volume of air is displaced from bottle A through the desiccator into the plastic bag. While the bag is filling a known amount of  $NO_2$  is added at the injection site. Valves  $V_2$  and  $V_3$  are then changed to allow diluted  $NO_2$  to flow through the animal exposure chamber,  $NO_2$  absorbing flask, and back into bottle A as water is siphoned into bottle B.

has been displaced from bottle A all valves are closed and the polyethylene bag is kneaded gently to assure thorough mixing of the gases. Mice are placed in the exposure chamber and the top is secured. Valves V<sub>2</sub> and V<sub>3</sub> are positioned to allow gas to flow into the exposure chamber and then through a gas washing flask for absorption of excess NO2 and back into bottle A. To start an exposure valve V<sub>1</sub> is opened allowing water to siphon into bottle B and drawing an equal volume of gas through the exposure chamber. The concentration of NO2 in the gas entering and leaving the exposure chamber may be determined after withdrawal of a measured volume of gas from the indicated sampling sites. The sample is injected into a serum bottle containing Saltzman's absorbing fluid. The syringe is rinsed several times with absorbing fluid before withdrawal from the bottle. The concentration of NO<sub>2</sub> is determined by the colorimetric method of Saltzman.<sup>2</sup>

# Discussion

Proportions of NO<sub>2</sub> in air are determined by the volume of gases allowed to enter the plastic bag. Except for dilution of the NO<sub>2</sub> by air initially present in the chamber the concentration of NO<sub>2</sub> during the exposure is constant and within 10% of the calculated value. Because of the changing head of pressure in the siphon, the gas flow must be adjusted intermittently with valve V<sub>1</sub> to maintain the desired flow rate.

A nearly constant concentration of NO<sub>5</sub> could be maintained over this short exposure period by the method used by Ripperton and Johnston. Several bottles of the gas mixture would be re-

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quired to reduce the chance of mixing the prepared NO<sub>2</sub> with air drawn into the bottle most removed from the chamber. The present system appears to be more convenient and it eliminates all chance of a changing concentration of NO<sub>2</sub> during the exposure period.

We are now experimenting with the use of a miniature positive displacement pump of the perastaltic type to deliver an intermediate concentration of NO<sub>2</sub> into a constant flow of air entering the animal exposure chamber. With this system the concentration of NO<sub>2</sub> should be independent of operating time and suitable for long term exposures.

#### Summary

A compliant plastic bag has been incorporated into a closed system designed for exposure of mice to nitrogen dioxide. The plastic bag allows the delivery of a constant concentration of gas at a controlled rate into an animal exposure chamber at nearly constant pressure.

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# REFINERY EMISSIONS

THOSE INTERESTED IN AIR POLLUTION problems in general and in petroleum refineries in particular will be interested in a new bulletin prepared by the
U. S. Public Health Service, Division of Air Pollution. Atmospheric Emissions from
Petroleum Refineries is a 56-page bulletin which describes the processes of the petroleum refinery, the types and sources of atmospheric emissions, and the applicable control methods. The publication is well illustrated with photographs, drawings, flow
charts, and tables. Methods, factors and examples are given for the calculation of the
atmospheric load of various emissions. The appendices also include an outline summary of control methods and a glossary of refinery terms.

This bulletin can be purchased from the Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C. Specify Public Health Service Publication No. 763. The price is 30 cents per copy.

#### MICROWAVES AND MEDICAL ELECTRONICS

THE ROME AIR DEVELOPMENT CENTER and the Tri-Service Committee on the Biological Effects of Microwave Radiation have, for the past four years, conducted an annual symposium on the biological effects of microwave radiation. This year, in order to promote the Fourth International Conference on Medical Electronics, the microwave symposium will be integrated with this Conference. A member of the Tri-Service Committee has been appointed to the technical program sub-committee and it is planned to have a plenary and special session devoted to the effects of microwaves, if suitable papers to support these sessions are received.

Papers which would normally have been submitted for the microwave symposium now should be sent to Dr. H. P. Schwan, Moore School of Electrical Engineering, University of Pennsylvania, Philadelphia 4, Pennsylvania. The theme of the Conference covers a broad scientific area common to the engineering, medical, and biological fields. Prospective contributors should submit an abstract of 300 words for preliminary review and a 50-word summary for inclusion in the advance program before April 1, 1961

The Fourth International Conference on Medical Electronics will be held at the Waldorf-Astoria Hotel, New York City, on July 16–21, 1961. Inquiries concerning registration, exhibits, program and other matters should be directed to: Fourth International Conference, Institute of Radio Engineers, 1 East 79th Street, New York, New York.

# Analysis of Chlorinated Hydrocarbons with the Gas Chromatograph

# PAUL URONE and JOHN ELVANS SMITH

University of Colorado, Boulder, Colorado

The qualitative and quantitative behavior of eleven chlorinated hydrocarbons on the gas chromatograph was investigated for possible application to the analysis of their vapors in air. Relative retention volumes and quantitative standardization curves are given. Methods for the sampling and measurement of chlorinated hydrocarbons in air are described.

THE remarkably high sensitivity and selectivity of the gas chromatograph has interested a number of researchers in using gas chromatographic techniques for the analysis of organic solvent vapors in air. Mansur and coworkers' developed a method of field sampling for organic solvent vapors. Matching gas chromatographic standards were prepared by similar treatment of known vapor-air mixtures from a gas chamber. West and co-workers<sup>2, 2</sup> have outlined methods of collecting and analyzing gaseous air pollutants. Levadie' has reviewed the work in this field and has outlined a method for direct injection of a sample collecting medium.

In studying the possibility of applying gas chromatographic techniques to the analysis of chlorinated hydrocarbon vapors in air, it became apparent that a more systematic study had to be undertaken. Although a number of workers had reported the chromatographic behavior of chlorinated hydrocarbons for theoretical as well as applied analytical reasons, 5, 6, 7 little of this information was directly applicable.

As a consequence, eleven chlorinated hydrocarbons were studied in some detail using nine column materials. The method used was to carefully measure the movement and thermal response characteristics of the compounds on the various column materials. After establishing the practicability of the qualitative identification and the feasibility of quantitative measurement of the amounts of chlorocarbons which could be accumulated from air samples, several alternate sampling methods were studied. The data obtained showed that gas chromatographic tech-

niques have considerable promise for the analysis of chlorocarbon vapors in air. Direct comparison of air sample chromatograms can be made with standards prepared from laboratory reagents without necessarily requiring use of a gas chamber or gas-air mixing systems.

A number of common non-chlorocarbon solvents were also studied principally for qualitative positioning on the chromatographic recordings

Figure 1 shows the chromatogram developed from 5 microliters of a mixture of the eleven chlorocarbons shown in Table I. The resolution is good with all eleven components being resolved in a 40 minute run. There is a slight overlap of methyl chloroform and 1,2-dichloroethane. If a mixture of these two were present in an actual sample, their peaks could be resolved more completely by different column conditions or by using a more suitable column material.

#### Instrumentation

For most of the studies, a Beckman Model GC-2 equipped with a wire filament thermoconductivity cell and a one millivolt, one second recorder was used. Most of the columns were prepared in the laboratory. They were generally composed of approximately 25% liquid phase and 75% chromosorb in six feet by one quarter inch copper tubing. The carrier gas was helium. In the later stages of the study, a Perkin-Elmer Model 154 C was used with approximately the same results (Figure 5).

#### Qualitative Identification

Unknown substances are generally identified by the time it takes for their peaks to be developed by the recorder following their injection into the chromatograph. More accurately, t g a a u t c

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Rochester, New York, April 25-28, 1960.

\* Presently with U. S. Naval Research Laboratory, Washington, D. C.

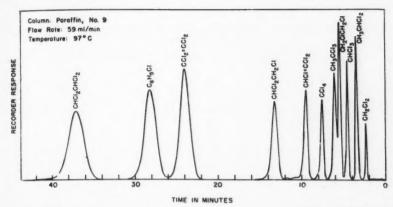


FIGURE 1. Chromatogram of eleven component mixtures on paraffin coated column.

the movement of a substance is measured by the volume of carrier gas needed to sweep the substance through the column. This volume is commonly called the retention volume. A properly corrected retention volume,  $V_{R}^{\circ}$ , can be calculated according to the following equation:  $V_{R}^{\circ} = f F_{c}t$ . Where f is the pressure gradient factor;  $F_{c}$  is the flow rate of the carrier gas through the base of the column at the column temperature, and t is the elapsed time from air peak to component peak. For experimental calculations, the following expanded relationships were used:

$$f \, = \, \frac{3}{2} \, \frac{(P_i/P_o)^2 \, - \, 1}{(P_i/P_o)^3 \, - \, 1} \, . \label{eq:force}$$

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$$F_c = F_m \frac{P_b - P_w}{P_b} \times \frac{T_c}{T_m}$$

 $P_i$  and  $P_o$  are the column inlet and outlet pressures, respectively;  $P_b$  is the barometric pressure;  $P_w$  is the water vapor pressure at the flowmeter temperature;  $F_m$  is the flow rate indicated by a soap bubble flowmeter;  $T_o$  is the Kelvin temperature of the column; and  $T_m$  is the temperature of the flowmeter.

A more convenient and popular method of representing retention volumes is by expressing the retention volumes relative to a common organic solvent which frequently is also used as an internal standard. For a given column, under a given set of conditions, relative retention volumes make unnecessary time consuming calculations. The use of an internal standard helps compensate slight variations in instrumental conditions.

Tables I and II list the retention volumes of the compounds studied relative to benzene. The compounds are listed in the order of increasing boiling points. In the paraffin, Apiezon L and Halocarbon columns, the retention volumes of the chlorocarbons increase with boiling point with the exception of 1,2-dichloroethane. With the tri-o-cresyl phosphate (TCP) and Carbowax columns some important reversals can be noted. These reversals are especially helpful in confirming qualitative analyses. Since there is always a possibility of coincidence of peaks on a given column, the much smaller possibility of similar reversals on different columns is of considerable help. The asterisks indicate compounds whose peaks will coincide with other chlorocarbons when injected simultaneously into the chromatograph.

A plot of the boiling points of the eleven chlorocarbons versus their retention volumes on a paraffin column gives a smooth curve with nine of the eleven compounds falling nicely on the curve. Two of them, 1,2-dichloroethane and 1,1,2-trichloroethane, exhibit low retention volumes. As a first approximation the expected retention volumes can be considered to be proportional to the boiling points of the compounds.

## **Quantitative Applications**

Figure 2 gives standardization curves for four representative chlorocarbons plotted as micromoles of chlorocarbon versus recorded peak height. The curves were developed by the injection of dilute solutions of the chlorocarbons in such solvents as toluene, isooctane and o-xylene. Based on a recognizable peak height of one tenth of an inch and using the maximum sensitivity of a one-millivolt, one-second Brown recorder the

TABLE I

Retention Volumes of Chlorinated Hydrocarbons Relative to Benzene = 1.00 on Various Columns at Two Different Temperatures

Compound	Boiling Point	Par	affin	Apiez	on L	Haloca	arbon	TO	P	Carbov	vax 4000	Carbo	owax 20M
Compound	°C	74°	97°	74°	97°	74°	97°	74°	97°	74°	97°	74°	97°
CH <sub>2</sub> Cl <sub>2</sub>	40.1	0.21	0.23	0.21	0.22	0.20	0.16	0.42	0.44	0.79	0.74	_	0.74
CH <sub>2</sub> CHCl <sub>2</sub>	57.3	0.37	0.40	0.37	0.38	0.40	0.36	0.54	0.55	0.62*	0.60*	-	0.61*
CHCl3	61.3	0.55	0.56	0.57	0.56	0.52	0.46	1.03	0.99	1.56	1.38	0.000	1.40
CH2CCl2	74.1	0.80	0.79	0.77*	0.79*	0.87*	0.83*	0.73*	0.74*	0.65*	0.65*	-	0.67*
CCl4	76.8	1.04	1.03	1.02	1.01	1.00	0.95	0.75*	0.76*	0.63*	0.64*	-	0.66*
CH2ClCH2Cl	83.5	0.69	0.69	0.72*	0.74*	0.82*	0.72*	1.43**	1.36**	2.19	1.98	-	1.95
CHCI=CCl2	87	1.39	1.33	1.44	1.37	1.44	1.27	1.36**	1.33**	1.38	1.32	-	1.32
CH2CICHCl2	113.5	2.10	1.97	2.34	2.18	2.49	2.16	4.70	4.17	7.92	6.31	-	6.27
CCl2=CCl2	121.0	4.18	3.67	4.27	3.82	4.39	3.81	2.70	2.46	1.86	1.81		1.86
CeHsCl	132	4.98	4.38	5.32	4.74	5.52	4.78	6.18	5.34	6.16	5.43	-	5.46
CHCl2CHCl2	146.3	6.86	5.83	7.91	6.77	7.79	6.58	22.97	16.69		25.21		24.89

\* and \*\* = Coinciding Peaks.

TABLE II

Retention Volumes of Some Common Solvents Relative to Benzene = 1.00 on Various Columns at Two Different Temperatures

Compound	Boiling Point	Paraffin		Apiezon L		Halocarbon		TCP		Carbowax 4000		Carbowax 20M	
	°C	74°	97°	74°	97°	74°	97°	74°	97°	74°	97°	74°	97
MeOAc	57.1	_	0.14(T) 0.23(T)	_	-	-	-	0.34(D)	0.35(D)	0.41	0.42 0.67	_	0.4
MeOH	64.7	_	_	0.09(T)	_	_	_	0.27(T)	_	0.62	0.56	_	0.5
EtOAe	77.2	-	0.47(T)	0.42(T)	-	_	-	0.65	0.63	0.64	0.63	-	0.6
Benzene	80.1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	1.0
"Isooctane"	99.3	1.50	1.45	1.22	1.18	1.22	1.20	0.37	0.39	0.14	0.15	-	0.1
Toluene	110.6	2.70	2.49	2.69	2.48	2.87	2.67	2.37	2.24	2.02	1.96	-	1.9
Me-Cellosolve	124		(HT)	(HT)	****	(HT)	-	2.71(T)	2.33(T)	5.09	4.40	-	4.1
n-BuOAe	126.5	-	3.22(T)	(HT)	-	(HT)		3.24	2.79	2.47	2.29	-	2.2
											3.13	-	2.9
p-Xylene		6.85	5.79	6.64	5.73	7.26	6.33	5.24	4.61	3.81	3.61	-	3.6
m-Xylene	139.1	6.93	5.84	6.73	5.80	7.90	6.64	5.42	4.78	3.98	3.74	-	3.7
Isoamyl-OAc	142.5	_	(HT)	(HT)	_	(HT)	_	3.64(Tr)	3.11(Tr)	2.38	2.21	-	2.5
								4.87	4.18	3.32	3.00	-	3.6
								7.11	5.67	4.59	4.00	-	4.0
o-Xylene	144.4	8.09	6.86	8.12	6.94	9.14	7.86	6.92	5.98	5.23	4.83	-	4.5

D = Double Peaks; T = Tailing; HT = Heavy Tailing; Tr = Triple Peaks.

minimum detectable amount for dichloromethane was 0.008 micromole or 0.7 microgram; for carbon tetrachloride, 3 micrograms; for trichloroethylene, 5 micrograms; and for tetrachloroethane 14 micrograms. This latter compound develops with a broad peak; hence it exhibits low peak height.

In making standardization curves, peak heights are most easily and conveniently identified and measured. However peak heights are most susceptible to slight experimental and instrumental variations. The measurement of peak

areas is less subject to experimental fluctuations and peak areas are frequently used for more exacting analyses. Figure 3 shows standardization curves plotted as micromoles versus peak areas. Strikingly noticeable is the fact that the standardization curves are nearly superimposable at low concentrations. The principal differences lie in the relative thermoconductivities of the vapors. Here again, as a first approximation one standardization curve would do for a number of possible unknowns.

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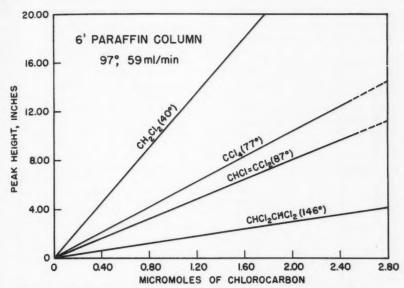


FIGURE 2. Standardization curves for representative compounds based on peak heights.

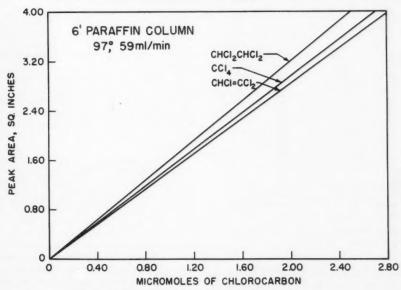


Figure 3. Standardization curves based on peak areas.

# Application to Air Sampling

Once the qualitative and quantitative properties of the chlorocarbons had been tabulated, attention was centered on possible air sampling

and recovery techniques. A small gas mixing chamber in which a microflask was placed and vented while a measured stream of purified air was passed through it was built. The concentration of the chlorocarbon vapors in the air was

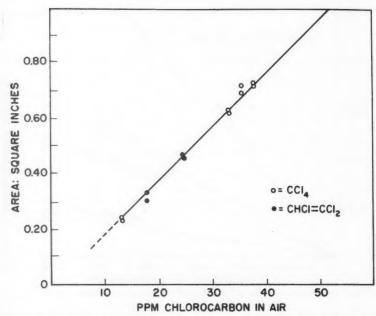


Figure 4. Peak areas obtained from 500 milliliter air samples using adsorption tube technique.

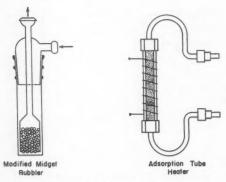


Figure 5. Devices used for sampling solvent vapor-air mixtures.

calculated by weighing the microflask before and after each run. Greater or lesser amounts of the chlorocarbons were vented by movement of the inlet air jet or by heating or cooling the flask. The incoming air was purified by a large silica gel drying column followed by "Molecular Sieve 13X" and magnesium perchlorate absorption tubes. Several capillary flow meters were used to measure the air, and each was calibrated by

either a wet test meter or by water displacement techniques.

A number of devices could be placed at the outlet end of the mixing chamber. One of these was a 40 liter "Mylar" plastic bag in which a given mixture could be stored and used at will. Alternately either a microbubbler or an absorption tube could be placed directly in line or in a bypass line.

Figure 4 shows two of the sampling devices used. On the left is a midget impinger converted into a microbubbler. As little as two milliliters of a scrubbing solvent are placed in the bubbler which is cooled in a dry-ice isopropyl alcohol bath. The air is passed through the bubbler in a direction reverse to normal and at approximately half a liter per minute for twenty to forty minutes. Loss of solvent by evaporation and spray is small and the efficiency of collection is 95% or better. A portion of the scrubbing solvent is injected into the chromatograph for qualitative and quantitative measurements. This method of sampling was reported by us in 195210 and has been used by others.1.4 It is very useful for composite air samples and for extended qualitative analyses including possible infrared studies.10

One of the disadvantages of using this method for chromatographic analyses is that only a small part of the scrubbing solvent can be injected into the chromatograph. If the whole sample could be injected into the chromatograph at one time, a much smaller sampling volume of air would suffice. This is possible if the air contaminants are first adsorbed on a solid adsorber and then desorbed into the chromatograph with heating or displacement. Such a technique has been described by West.2 The device worked out for this general method is shown on the right in Figure 4. It consists of a special heater which is attached to the gas sampling valve of a chromatograph. As little as 200 milliliters of air is sampled by a small tube containing a solid adsorbent. The adsorption tube is placed in the heater and heated for a precalibrated time of one to two minutes. The sample is driven into the helium gas stream and through the chromatograph.

Figure 5 illustrates results obtained using this type of technique. Five hundred milliliters of known concentrations of chlorocarbons were drawn through a silica gel adsorption tube at room temperature. The silica gel was previously conditioned by heating in the presence of helium and was protected from water vapor by a short tube of magnesium perchlorate. 10 After sampling. the adsorption tube was inserted in the heater. Helium was allowed to flow while an electric current was applied with just sufficient voltage to raise the temperature of the tube to 250°C in two minutes. The straight line relationship between concentrations and area of peaks holds at least up to 100 ppm. The area of the peaks are essentially independent of the type of chlorocarbon present.

# Conclusions

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hol in oxito cion lecing for Chis 5210 eful ded Gas chromatographic techniques afford a simple, sensitive, and selective method for the analysis of chlorinated hydrocarbon vapors in air. Higher resolution with better qualitative sepa-

rations are obtained with paraffin coated columns. As a first approximation, paraffin columns elute chlorocarbons in the order of their boiling points.

Quantitative measurements may be made from peak heights or peak areas. However, peak areas vary only slightly with the type of chlorocarbon and may be used independently of the type of chlorocarbon being measured. As little as 200 milliliter samples of air passed through activated silica gel capsules will give measurable peaks when desorbed into a gas chromatograph by means of a heating tube attached to the gas sampling valve of a gas chromatograph.

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# Light-Scatter Instrumentation for Measurement of Atmospheric Particulates

# JOHN S. NADER, GORDON C. ORTMAN, and MORGAN T. MASSEY

Air Pollution Engineering Research, Robert A. Taft Sanitary Engineering Center, U. S. Public Health Service, Cincinnati 26, Ohio

The determination of mass and count measurements of particulates in air by use of light scattering instruments is discussed. Operation and performance of the Monitron and a miniturized, portable instrument are described, and data on comparisons with other instruments are given.

ATMOSPHERIC particulates consist of solid particles and liquid droplets ranging in size from millimicrons to tens of microns and varying in shape from spherical fog droplets to porous and irregular soot particles. Their source could be natural, the result of condensation, dust storms, and forest fires or artificial, resulting from the labors of man and industry. Particulates are considered pollutants inasmuch as they can reduce visibility, cause property damage, and produce toxic effects both in man and plant life. As man-made pollutants, they are subject to abatement and control. Instrumentation is necessary, therefore, to evaluate the contribution of man-made particulates to the air pollution problem, the nature of various particles and their correlation to specific effects, and the effectiveness of specific control measures.

Instrumentation for the detection and measurement of particulates can operate according to any one of a number of principles which relate to the nature of the particle. For example, the mass of a particle will allow for inertial collection and analysis; the charging property for its electrostatic detection; its radioactivity for its nuclear-measurement; and its optical properties for light scatter measurements.

Collection and analysis of such small particles has been known to present some difficulties. Low particulate concentrations require long sampling periods to collect a sample which could be weighed to give mass concentration. Such concentrations, then, only represent an average value for a long time interval but give no measure of short term variations. The sampling method takes into consideration filtering effi-

ciency, air flowrate, and sampling medium which must meet the requirements of the subsequent method of analysis. Some of the analytical methods of microscopy, gravimetry, or photometry may emphasize aspects of sampling that are contradictory. A gravimetric sample needs to be large for accurate weighing. A sample for microscopic analysis, on the other hand, has to be fairly light to distinguish individual particles for particle sizing or chemical spot tests. Furthermore, the need for collection of a sample prior to analysis provokes a question which will most often be asked, namely, how much of an agglomerating effect is there in any given sampling technique? Or more simply, how representative is the collected sample of its prior existence in the sampled air?

Photoelectric measurement of light scattered by particulate offers a sensitive method of analysis without some of the drawbacks of sample collection. The method is sensitive in the particle size range of immediate interest, from 0.2 to 2  $\mu$  diameters. Particles in this size range are, to a great extent, retained in the lungs and can be a potential health hazard. Furthermore, since particles in this size range have diameters near the wavelength of visible light, they have optimum visible light scatter characteristics and the undesirable effect of reducing visibility.

#### Mass and Particulate Concentration

Early photoelectric devices were developed to measure relative particle concentrations in the evaluation of smoke respirators and filtering media. A sensitive method by Gucker, et al. consisted of a measurement of light scattered by the particulate in an air stream at right angles to the path of the incident light beam. This method was satisfactory as long as the size distribution

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remained constant and the objective was measurement of the relative concentration of two samples of particulates of the same size. It had the disadvantage in that measurements were affected by any change of light intensity between the calibration with a test aerosol and the measurement with the sampled aerosol. In addition, measurement of absolute instead of relative concentrations required standardization with a known aerosol concentration.

Subsequently, Peterson's developed a differential photometer which used the optical system developed by LaMer and Sinclair. Dark-field illumination was used to illuminate the sampled particles which scattered light in the nearforward direction (between 5 and 35° from forward) onto a photomultiplier. The photomultiplier permitted increased sensitivity and stability over the vacuum photocell and associated electronics of the simple right angle scatter instrument. This improved photometer also compensated for any change in light intensity or photomultiplier sensitivity by using a portion of the incident light beam as a reference beam which is periodically monitored by the photomultiplier through a rotating shutter. Any deviation of the photomultiplier output from a preset level due to changes in light intensity or phototube gain was amplified and activated a motor which varied dynode voltage on the pho-

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tomultiplier and thus brought about a change in sensitivity and the necessary compensation.

A right angle light scatter photometer was later developed by Mendenhalls who used essentially the same concept of splitting the incident light beam to give a reference monitor beam which automatically compensated for changes in incident light intensity or photomultiplier gain. The dynode voltage in this case was varied by negative electronic feedback instead of electromechanically by a servomotor drive.

The performance of the "Monitron" (Engineering Specialties, Madeira, Ohio) a commercial version of Mendenhall's development, is currently being evaluated as a continuously monitoring device and in conjunction with other related measurements of mass particulate concentration in the atmosphere. The optical system (Figure 1) and the electronic system (Figure 2) follow essentially the prototype version. The light from a six-volt, automobile headlight bulb is split into two beams, the monitor beam,  $I_m$ , and the incident beam,  $I_4$ . Both beams are alternately interrupted by an arrangement of holes in the rotating light chopper disc. The monitor beam is reflected into the photomultiplier by several 45° mirrors, one of which has an adjustable angle of reflection so that the intensity of the monitor beam reaching the photomultiplier can be increased or decreased. The point of right angle intersection of the incident beam

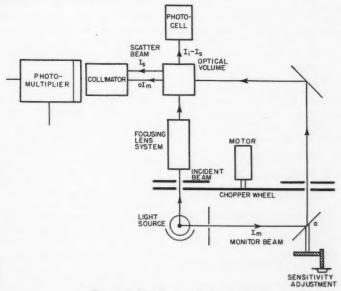


FIGURE 1. Monitron optical system.

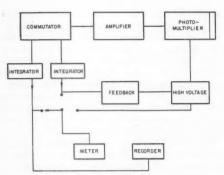


FIGURE 2. Monitron electronic system.

and the path of view of the photomultiplier is the "optical volume" which is in an isolated compartment of the instrument and thru which sampled air is pumped by a squirrel cage blower having an air flowrate of approximately 6 cfm. The air flowrate can be decreased by reducing the size of the exhaust outlet. Particles present in the optical volume will scatter light,  $I_{\bullet}$ , from the incident beam onto the photomultiplier. Consequently, the photomultiplier will have a pulsed output signal for the monitor beam 180° out of phase with the pulsed output signal for the scattered light beam. The two signals are separated by the commutator which is attached to the shaft of the light chopper. Each pulsed signal is integrated by a small time constant (0.5 second) circuit which provides a D.C. signal to the meter. By feedback of the monitor output signal, the overall gain of the instrument (i.e., photomultiplier sensitivity and light source intensity) is automatically adjusted to maintain a constant monitor signal output, Rm, against which the scatter light signal is referenced.

A better understanding of the interpretation of the meter reading,  $R_s$ , for the scattered light, in terms of the light scatter coefficient,  $\sigma$ , can be shown briefly as follows:

$$R_* = GSI_* = GAc\sigma I_* \tag{1}$$

$$R_m = GAaI_m = GAabI_o (2)$$

where  $R_m = \text{Meter reading of monitor beam, a}$ constant

 $R_* =$ Meter reading of scattered light beam

A = Amplification factor of amplifier and a constant

a = Coefficient as function of sensitivity setting

I<sub>o</sub> = Light source intensity
 I<sub>s</sub> = Scattered light beam

Im = Monitor light beam

 $b^m = I_m/I_o$ 

 $I_{i} = \text{Incident light beam}$ 

 $c = I_4/I_o$ 

 $\sigma$  = Light scatter coefficient,  $I_s/I_s$ 

 $G = Gain ext{ of photomultiplier, which}$ because of negative feedback is an inverse function of  $I_m$  or  $bI_a$ .

$$R_s = (R_m/ab)\sigma = K\sigma \text{ where } K = R_m/ab (3)$$

The results in Equation (3) show that  $R_*$  is directly proportional to the scattering coefficient. Furthermore, for a given scattering coefficient,  $R_*$  is a function of the sensitivity setting or an inverse function of the intensity of the monitor beam as seen by the photomultiplier.

Minor modifications were made on the Monitron in our laboratory as the need arose. These consisted of the addition of a reflector on the light source to increase the scatter signal (by factor of 4); addition of a photovoltaic cell at the end of the incident light beam to observe relative changes in source intensity; the installation of a manually operated shutter to block the incident light beam for a measure of the dark current response of the photomultiplier; and the installation of a light scatter probe which can be manually introduced into the optical volume as a test reference for the overall response of the optical and electronic system.

# Particle Count and Size Distribution

Most of the important effects of atmospheric particulate in the study of air pollution, such as reduced visibility, soiling, corrosion, control, etc., are strongly dependent upon the particle size and number. Hence, measurement of these particle parameters is of importance in characterizing their presence.

Again, the history of the development of the particle counter shows that a counter based on light scattering was developed (Gucker, et al.) to provide ultra-sensitive test of gas-mask service canisters by determining the penetration of single aerosol particles slightly larger than 0.3 µ diameter used in penetrometer tests. This was followed by a second counter developed for counting and measurement of particle size by pulse discrimination. Subsequent developments became more complex with the use of multiple channel analyzers and associated readout accessories. The size alone of the composite instrument for particle count and size analysis limited its use to a fixed installation with controlled aerosols. A study of the particle parameters of atmospheric particulate, however, required a field type instrument which would be portable 1

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with a self-contained power supply. To meet this need, the U. S. Public Health Service contracted for and has received delivery of a miniaturized light scatter photometer and pulse height analyzer for field measurements of particle count and size distribution. (Developed under Contract No. SAph 78964 for the Robert A. Taft Sanitary Engineering Center, U. S. Public Health Service.) The instrument has been designed to make these measurements of aerosols in the atmosphere and of solid particles or liquid droplets in gases. The instrument is portable and battery operated, employing transistor circuitry. The counter measures 14½ x 5 x 8% inches and weighs 10½ lbs.

The operation of the instrument is as follows: Air is drawn through the flow system at a rate of 17 cc/min by a small positive displacement gear pump. Dilution air is obtained from the environment through a high efficiency filter. The dilution valve gives the desired ratio of dilution to sample air. This can be set from zero dilution (totally sampled air) to infinite dilution (totally filtered air) and calibrated at intermediate dilution factors of 5, 10, and 20. Filtered air surrounds the sample air stream to prevent dispersion of particles in the photometer head and to maintain laminar flow of the sample stream through the optical volume. For concentrations within full scale range, particles are viewed one at a time as they are drawn into the illuminated optical volume of 0.5 microliters. The scattering angle is 135° from the incident light beam.

The photomultiplier tube produces an electrical pulse of approximately one millisecond duration from the light scattered by each particle. These pulse amplitudes are analyzed for electrical size distribution and displayed in terms of particle size diameters, based on calibration with monodisperse polystyrene spheres. Pulses produced at the phototube are amplified and applied to the one discriminator of the particle counter and to each channel discriminator of the particle size analyzer. The discriminator output pulses are then amplified and counted. An electrical signal proportional to the logarithm of the number of pulses is indicated on the meter of the particle counter; similarily, a channel signal is used to drive each galvanometer of the pulse-height analyzer.

For manual size analysis the particle counter may be operated independently to obtain a count of particles above a given size. The cut-off is adjustable from 0.3 to 2 microns by a manually operated discriminator. A series of readings at various cut-off sizes will give a cumulative distribution of particle count vs. size. Automatic size analysis in the range from 0.3 to 10 microns

is obtainable by operation of the particle counter in conjunction with the 12 channel pulse-height analyzer. A size distribution histogram of the analyzer output is displayed on the screen of a multi-channel galvanometer. There are also simultaneously displayed the values for the dilution factor and the total particle concentration. The entire display may be "frozen" at any instant to permit photographing the information.

Particles may be counted at a rate of 10 and 100 per second corresponding to an undiluted concentration of one and ten million particles per cubic foot, respectively. For a dilution factor setting of 10 these correspond to 10 and 100 million particles per cubic foot, respectively. Particle count may also be accumulated over a period of time for concentrations less than one million particles per cubic foot. Maximum reading time is two minutes which gives a full scale value of 0.5 million particles per cubic foot. With the log scale this gives a minimum readable concentration of five thousand particles per cubic foot.

#### Field Measurements and Discussion

As part of a preliminary study initiated to observe what correlation may exist between various air quality measurements of particulates, a number of particulate measuring instruments were operated for the greater part of March at the Gest Street Experimental Facility of Air Pollution Engineering Research, Robert A. Taft Sanitary Engineering Center. The Gest Street Facility is located in an industrial environment close by the downtown area of Cincinnati and is instrumented to measure meteorological parameters and to obtain various air quality data. Operating concurrently at various times with the Monitron were the following instruments: Transmissometer which measured the attenuation of light by atmospheric particulate over a 750 foot path length and whose data is expressed in miles of visibility; membrane tape samplers for subsequent analysis of soiling and mass loading by particulates; portable particle counter and size analyzer.

Particulate material collected on membrane tape° for four-hour intervals was analyzed using the mass loading analyzer¹⁰ to express the data in terms of average mass concentration per unit volume for the specified time interval, i.e., micrograms per cubic meter. The analyzer used a beta gauge technique to measure the thickness of particulate deposition and was operating near its limit of sensitivity to detect the small thicknesses of particulate deposits (<0.1 mg/cm²) collected in the four-hour interval. Particulates

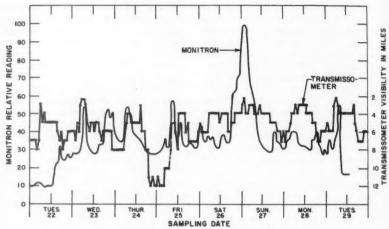


FIGURE 3. Comparison of Monitron and Transmissometer data.

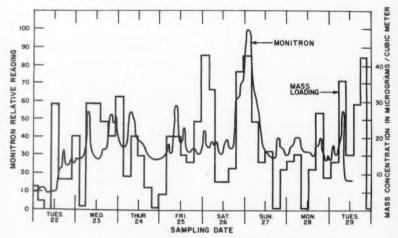


FIGURE 4. Comparison of Monitron and mass loading data.

also collected on membrane tape for two-hour intervals were analyzed by light transmission measurement of the particulate depositions."

Data obtained over a period of one week in March during which all the instruments were operative were graphically evaluated for comparisons of various air quality measurements. The curves of one-hour average values for the Monitron's light scatter readings and the Transmissometer's visibility readings show some graphical correlation in the rise and fall of both values (Figure 3). This is what one might expect considering that both instruments operate on light scatter principles. It is well known, how-

ever, that light scatter from natural pollutants such as fog plays a significant role in Transmissometer measurements. Additional study of the data will be necessary to observe what effect high humidity has on the Monitron measurements and the possibility of reducing this effect by some technique such as preheating the sampled air. Further evaluation of the data may indicate that the correlation is sufficiently high to permit calibration of the Monitron in terms of visibility readings. Such an approach will provide a practical and convenient means of instrumenting visibility measurements.

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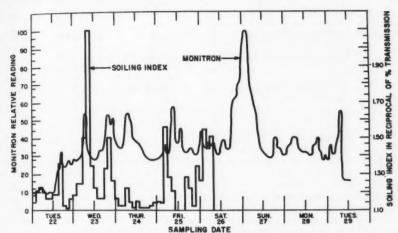


FIGURE 5. Comparison of Monitron and soiling index data.

concentration and light scatter values (Figure 4), would also indicate the possibility of empirical calibration of the Monitron for measurement of mass concentration of atmospheric particulate, thus eliminating the need for sample collection and providing a sensitive measure within a shorter time interval. Additional work will be necessary, however, in which monodisperse polystyrene spheres of known sizes, as well as representative atmospheric particulate samples, are used to obtain information on the effect of various particle diameters and size distribution and particle number on mass loading calibration.

Data on the soiling index were limited to part of the period of interest due to malfunction of the sampler later in the week (Figure 5). There are indications of some correlation which will have to be looked into further.

The particle size analyzer was operated on several occasions during this period and the size distributions obtained showed a predominance in the number of particles below two microns and a peak in the distribution somewhere below 0.3 microns. Considering that the atmospheric content of particulates is a composite of particles from different sources more or less in equilibrium, it is very likely that this distribution will be representative until a specific source of high output has a drastic change in its contribution.

The light source of the particle counter had a relatively short life of about 17 hours and maintained a fairly constant intensity without adjustment for about two to four hours. As a result it did not lend itself to continuous unattended monitoring over any length of time. Data were obtained, however, concurrently with the other

air quality measurements to permit a calibration in terms of particle count.

All the graphical data shown are being statistically evaluated to obtain correlation values which should provide a reliable quantitative measure of the graphical interpretation. Additional field data will be required to substantiate these preliminary findings on the use of light scatter values as a measure of visibility, mass loading, and soiling parameters of atmospheric particulate.

# Acknowledgment

The authors express their appreciation to staff members, Mr. Eugene Cordier, Physicist, Mr. William Coffey, Electronic Engineer, and Mr. Charles Norris, Electronic Technician, for their assistance in this study.

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## AIR CLEANING INSTITUTE

HARVARD University School of Public Health will present an intensive course on air cleaning, June 19-23, 1961.

The course will consist of lectures and laboratory sessions which have been designed to provide graduate level training in theoretical and applied aspects of air and gas cleaning procedures and equipment. Preliminary or refresher sessions will afford a review of basic engineering principles in order that coverage of advanced material may be as comprehensive as possible. Topically, the course will include:

- A. Basic Theories and Principles of Aerosol and Gas Collection: particle dynamics and behavior.
- B. Stack Sampling Methods and Data Evaluation: loading, particle size, efficiency.
- C. Performance of Typical Air & Gas Cleaners: efficiencies, costs, applications to special industrial hygiene and air pollution problems.
- D. Laboratory Demonstrations: illustrations of lecture material.

Student enrollment will be restricted to those actively engaged in the practice of industrial hygiene engineering or air pollution control. An academic background consisting of engineering or physical sciences is preferable but not a prerequisite provided that the candidate has a minimum field experience of 8 years and a need for special training. Total enrollment for the course is limited to 40 persons due to the specialized nature of the laboratory requirements.

Tuition for the course will be \$150.00. A registration free of \$50.00, which must be submitted with each application, will be credited toward tuition payment if the candidate is accepted; otherwise, the fee will be returned. Applications will be processed by the Admissions Committee according to the date received. No application will be considered unless accompanied by the registration fee.

For further information write to: Leslie Silverman, Sc.D., Harvard School of Public Health, 55 Shattuck Street, Boston 15, Massachusetts.

# Contaminant Generators for Continuous Exposure Inhalation Chambers

Lt. (jg) ROBERT V. FULTYN, MSC, USN

U. S. Navy Toxicology Unit, National Naval Medical Center, Bethesda, Md.

The design of a mist generator for continuous operation for prolonged periods of time with animal inhalation exposure chambers is discussed. Operating tests showed it to be capable of maintaining satisfactory uniform concentrations of aerosol in the exposure chambers with a minimum of attention.

#### Introduction

THE advent of the nuclear submarine has brought about the requirement of exposing men to industrial type atmospheres for longer periods than were heretofore necessary. With these long term exposures there is a need to investigate not only the toxic properties of new air pollutants, but also to reevaluate the hazards of the older and more familiar substances under these new conditions of prolonged exposure.

In order to conduct investigations to assist the Medical Department of the U. S. Navy to evaluate the toxicity of various air pollutants on the basis of 24-hour-per-day exposure for periods of several months, the U. S. Navy Toxicology Unit (NTU) was established at the National Naval Medical Center in Bethesda, Maryland. The equipment provided to conduct long term studies of exposure by inhalation includes a group of six stainless steel and plexiglass exposure chambers identical to those described by Leach et al.' and presently in use at the University of Rochester for chronic studies with uranium oxide dusts.

#### Chamber Arrangement

The Rochester arrangement has been modified somewhat for use at NTU. At NTU the chambers are grouped in three pairs, a typical pair shown in schematic in Figure 1. Air is drawn from outside into an air conditioner which serves both chambers of the pair. The conditioned air is directed to the immediate vicinity of the chambers by a main supply duet, and then into the chambers through individual ducts. Just before entry into the chamber the contaminant is introduced into the air stream. Each chamber is provided with its own contaminant

dispersal generator. Before leaving the chamber the air must first pass through a rough filter at the entry of the exhaust duct, which removes larger matter such as hair and food dust. The exhaust air is then directed to a filter house where it is further cleaned, then through the exhaust blower and outside. Each chamber is provided with its own filter house and exhaust blower. The exhaust blower is the prime mover of air through the system, and air flow can be controlled by adjusting blower speed and by means of dampers. The air flow through the system is measured by means of Gentile Flow Tubes, Type VDM, manufactured by the Foster Engineering Division of General Controls Company. These flow metering devices may be inserted either into the inlet or exhaust lines of each chamber.

In order to minimize the interruptions in the exposure of the animals which must be kept in the chambers at all times, but still to keep the chambers as clean as possible, an internal wash system was installed. This system consists of a wash ring installed around the edge of the chamber, just below the lower floor.

In order to meet the requirements of continuous exposure without the use of a very large staff, devices capable of being automated to give reliable performance with a minimum of attention were desired. In addition to this it was very desirable to have devices which would be as versatile as possible, so that they could be used with a wide variety of substances that might be investigated.

#### Design of Mist Generator

The first substances to be studied were oil mists, and most attention has been directed toward development of a mist generator.

Many excellent mist generating devices were considered and rejected either because of the difficulty and expense of regulating them auto-

Presented at the Twenty-first Annual Meeting of the American Industrial Hygiene Association, Rochester, New York, May, 1960.

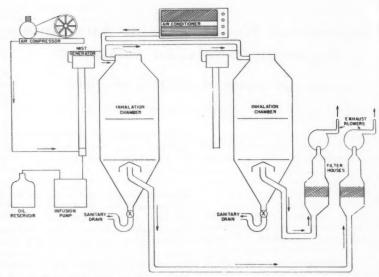


Figure 1. Schematic diagram of one pair of exposure chambers at U. S. Navy Toxicology Unit.

matically, or because they employed physical principles which would limit their ultimate versatility.

A device utilizing a spray nozzle was selected because of its inherent versatility. It can be used to create mists of a wide variety of substances. In general, nozzles cannot be expected to produce mono-disperse aerosols, but this was relegated to secondary importance in view of the fact that mists produced as a result of industrial operations are likewise seldom monodisperse.

A nozzle of the two-fluids type was selected in preference to a single fluid nozzle because good atomization over a wider range of liquid flow rates would be easier to attain using the two-fluid principle. The final choice was an available modification of a paint spray gun nozzle which produced particle size characteristics a desirable as any investigated, with the added advantage that it could be rapidly and easily disassembled for cleaning and reassembled.

Under most conditions of operation with nonevaporative liquids the spectrum of particle sizes produced by this nozzle contains a very large proportion of aerosol which would not be expected to penetrate very deeply into the respiratory systems of the exposed animals. In order to remove these larger particles a single stage impactor was chosen. The design now used, shown in Figure 2, was chosen on the basis of preliminary investigations at another government installation. The nozzle is located at the bottom of and sprays upward into a long "laminar flow tube." At the top of this tube is a single stage impactor, consisting of the upper nozzle and impaction surface. Surrounding the single stage impactor is a drum, into which the aerosol can expand after impaction. From this drum the aerosol flows into the chamber main air suppy just prior to entry into the chamber. The generator is used in the vertical position, as indicated in Figure 2, for considerations of space and convenience.

The impaction theory presented by Ranz and Wong<sup>2</sup> permits the performance of an impactor to be predicted from the parameters of the system. This theory relies upon streamline flow through the nozzle, and for this reason the dimensions of the generator were chosen to achieve this condition as much as possible. The model used in this study was constructed of plexiglass. The laminar flow tube was four inches in diameter and 84 inches long, dimensions which should ensure laminar flow just prior to the upper nozzle for air flow rates through the impactor of up to three cubic feet per minute. The upper nozzle was nine inches long, tapering to an orifice 3/8 inch in diameter. The distance from the upper nozzle tip to the impaction surface was approximately 3/16 inch. The upper drum was 18 inches in diameter and 12 inches deep.

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The problem of keeping the impaction surface clear to avoid reentrainment of large drops was solved in the following way: The impaction surface was a 100 mesh screen, covering one end of a circular cylinder tightly packed with cotton wadding. The other end of the cylinder was capped, the cap being fitted with a hose connection which in turn was connected to a source of vacuum by means of a drain line. In operation, air is slowly drawn through the packed cylinder. As particles impinge upon the screen, they agglomerate, and are drawn with the air into the cotton wadding. After the cotton becomes saturated, the liquid trickles through the screen drain line. The impaction surface cylinder is held in position by means of a clamp and collar in the top of the drum. This arrangement also allows the distance from the upper nozzle tip to the impaction surface to be varied.

Drains are also provided at the bottom of the drum, and bottom of the laminar flow tube. In practice, the three drains are connected to a common manifold, and suitably constricted to achieve a desired balance of flow through each one.

# **Constant Operating Conditions**

Clean, oil-free air for the mist generator nozzle is supplied by a carbon ring compressor. Uniform delivery is ensured by the use of two stages of pressure regulation. Uniform, metered liquid flow is ensured by the use of a Harvard Apparatus Company Model 600-950 Continuous Infusion Withdrawal Pump (Figure 3). This pump consists of a pair of medical syringes uniformly operated by a synchronous motor, variable speed transmission, and worm drive. A valve arrangement is provided so that as one syringe is delivering liquid, the other is filling. At the end of each stroke the action automatically reverses, and provides a uniform, uninterrupted flow of liquid. The pump can be fitted with six different sizes of syringes, and has a twelve-speed transmission, allowing a choice of seventy-two flow rates from 0.00079 cm<sup>3</sup>/min to 38.2 cm<sup>3</sup>/min. Pump speed can be changed instantly by means of a shift knob.

Because the nozzle chosen frequently acts like an air ejector, the pump is often operating under a negative pressure. To reduce air leakage into the syringes around the plungers, the syringes have been fitted with rubber "boots", as shown in Figure 3. With this arrangement, accumulation of air in the syringes averages approximately 0.1 of a cubic centimeter per day. This allows operation for periods of a week at a time without necessity for removing air from the

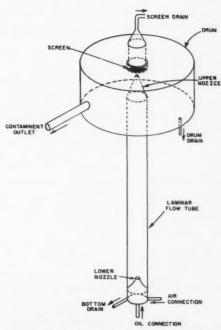


FIGURE 2. Diagram illustrating the features of the Mushroom Impactor Mist Generator.

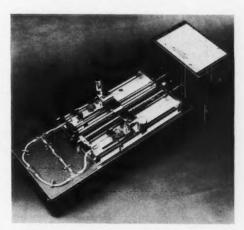


FIGURE 3. Continuous Automatic Infusion-Withdrawal Pump used for precise metering of liquid flows (Harvard Apparatus Co., Model 600-950).

syringes, and the amount of air accumulated in this time has had no noticeable affect on performance to date.

For conditions of constant operation, the

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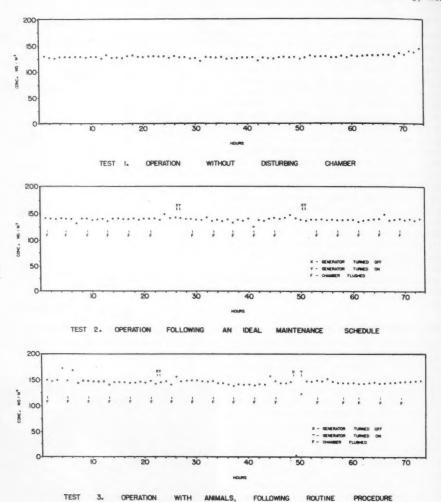


FIGURE 4. Results of experiments to determine the uniformity of aerosol concentration in NTU exposure chambers.

TABLE I Statistical Summary of Results

Test Number	No. of samples used	Mean aerosol concentration mg/M³	Standard deviation mg/M <sup>3</sup>
1	73	129	3.46
2	73	139	3.21
3	71	147	5.42

pump inlet is connected to a liquid reservoir of one gallon capacity, and the outlet to the generating nozzle. The drains from the mist generator are manifolded back to the same reservoir. The reservoir is kept under a slight negative pressure, which ensures adequate flow through the drains, but does not influence metering pump operation.

# Mist Generator Performance

This paper will attempt to illustrate how well this mist generator fulfills the objective of maintaining a uniform aerosol concentration for extended periods of time with only a minimum of attention.

The uniformity of concentration in a repre-

sentative chamber was tested under three condi-

1. Operation of the empty chamber without disturbing it except to regulate air flow through the chamber as necessary.

2. Operation of the chamber without animals, but following an ideal daily procedure to observe the effects of opening it for a twenty minute period each day, and washing out the bottom every four hours, using the installed wash system.

3. Operation with animals, allowing the staff to perform all regular activities in their routine fashion.

The substance used for the test aerosol was a synthetic safety type hydraulic fluid of current interest. Oil flow and high pressure air to the generating nozzle were maintained at constant settings throughout the tests, at values which were expected to give chamber concentrations of approximately 140 mg/m3. Air flow through the mist generator was 1.9 scfm. Air flow through the chamber during the first test condition was 43 cubic feet per minute. For the other two test conditions, air flow was 40 cfm. Chamber pressures were held at about 0.6 inch of water. Microscopic examination of aerosol samples taken with a Casella Cascade Impactor showed a maximum particle size of six microns, with the mass median particle diameter estimated to be two

Chamber concentration was sampled hourly for 72 consecutive hours under each test condition. Samples were collected on 2-inch diameter organic-free glass filter papers, and the amount collected was determined gravimetrically. Preliminary experiments showed that the gravimetric method would give sample weights accurate to within 1%. Filter holders were plugged into a rigid metal tube, one inch in diameter and 20 inches long, installed through the chamber wall. The position was fixed so that the point of sampling was eight inches above the tops of the upper animal cages, and seven inches from the chamber wall. Tests to determine the background dust concentrations in the chamber without animals showed an unmeasurable concen-

tration by this method. With animals in the chamber the background of weighable material fluctuated from 0 to 4 milligrams per cubic meter.

Figure 4 shows graphically the results of the tests conducted under the three types of operating conditions. The plots of test 2 and test 3 include indications of the times at which the mist generator was turned off and on, as well as the times at which the wash system was operated. Table I summarizes the values of the mean and standard deviation for each test.

#### Summary and Conclusions

To conduct investigations of the toxicity of air pollutants on the basis of a 24-hour-per-day exposure, contaminant generators suitable for continuous operations over long periods of time were developed for use with animal inhalation exposure chambers.

Tests conducted with a mist generator showed that it was capable of maintaining satisfactorily uniform concentrations in the exposure chambers with a minimum of attention for the periods herein investigated.

The suitability of this generator at other concentration levels, and for the productions of aerosols of different materials is currently under investigation.

#### Acknowledgments

The author acknowledges with appreciation the suggestions and encouragement given by Lt. Arthur J. Getzkin, MSC, USNR, and Dr. Seymour Friess. To the members of the staff of the U. S. Navy Toxicology Unit who aided in the collection of data the author expresses his sincere gratitude.

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# Isolation of Proteins from Samples of Airborne Particulates

VELTA GOPPERS, M.S. and HAROLD J. PAULUS, Ph.D.

School of Public Health, University of Minnesota, Minneapolis, Minnesota

Development of a method for protein extraction from a sample of airborne particles is discussed. The nature of the extracted proteins was investigated by means of electrophoresis. Electrophoretic patterns of extracts for different air samples are shown. Fourteen amino acids were identified following hydrolysis of proteins obtained from an air sample. Total protein determinations were made using two methods.

#### ntroduction

CTUDIES on the cause of a higher than normal incidence of asthma among students at the University of Minnesota have been undertaken. Adjacent to the campus and main student residential areas are grain mills and plants which emit air contaminates from various operations and processes. Student exposures to different kinds of particulates, gases and mists from the grain industry have been suspected as contributing to the frequent cases of asthma treated at the University Health Service. Grain proteins are among the materials being considered as causative agents for the precipitation of attacks of this disease. This paper describes the determination of total proteins, identification of amino acids and the separation of specific proteins from samples of airborne materials.

## Sample Collection

Airborne particulates were collected by means of a General Metals High Volume Sampler with a filter holder capable of accommodating a rectangular 8- by 10-inch filter. Samples were obtained in the campus and mill areas using a fibre glass filter. A 24 hour sample at a sampling rate of 50-60 cubic feet per minute provided a sufficient quantity of material for protein analysis and separation procedures. Special samples of airborne material were collected in the mills during grain unloading and other operations. Collection was made on a 2-inch fibre glass filter using an air ejection suction device since the use of electric equipment was prohibited. For certain laboratory experiments, bulk samples of different grain brans were obtained at unloading

operations in the mills. These samples contained some particulates that were relatively large in size and would not ordinarily be airborne. In

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#### **Total Protein Determination**

Two methods were used for estimation of total proteins, one being a micro-Kjeldahl nitrogen method¹ modified for fibre glass filter samples and the other an amino acid determination as described by Chambers et al.²

Preparation of the samples for total protein analysis was accomplished by an 8-hour acetone extraction of the particulate matter on the fibre glass filter. According to Chambers this procedure removes the non-protein organic matter with only a nominal protein loss. It was found in our work that the elevated temperature of extraction did change the protein molecules by splitting off amino acids.

The extracted material was separated into two equal portions and total proteins determined as described below.

#### Micro-Kjeldahl Method

Crystalline egg albumin with a nitrogen content of 15.28% was used for preparation of the standard protein curve. Solutions containing known amounts of egg albumin and 150 milligrams of clean fibre glass filter (previously extracted with acetone) were digested with 4 ml of sulfuric acid (1:1) plus one grain of Hengar granule. Digestions were continued until all nitrogen was converted to ammonium sulfate, which required from 20 to 30 minutes. Then 150 milligrams of the acetone extracted fibre glass filter containing from 10–20 milligrams of particulate matter including unknown proteins were treated in the same manner. The cooled, digested mixtures were diluted with 5 ml of distilled was

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ter, (throughout these procedures only nitrogen free distilled water was used) filtered and the residue washed. The clear filtrates were steam-distilled with 20 ml of 4N sodium hydroxide to liberate the ammonia. Distillate was collected in 20 ml of 0.1N sulfuric acid placed in a 50 ml volumetric flask. Steam distillation was continued until about 48 ml were collected, the condenser tube rinsed with distilled water, and the solution diluted to 50 ml.

The amount of ammonia was determined by a colorimetric method using Nesslers reagent. One ml of each distillate was transferred to a 10 ml volumetric flask and diluted approximately to 5 ml with distilled water. One ml of Nesslers reagent was added, then distilled water up to the mark. Colors were compared against standards in a Spectronic colorimeter.

#### Amino Acid Method<sup>2</sup>

The airborne particles containing proteins, after extraction of the interfering organic materials, were hydrolyzed for 20 hours with 6N hydrochloric acid, diluted to a known volume and centrifuged. The following steps were then used: a measured volume was evaporated to dryness, residue dissolved in distilled water, pH adjusted to 8.4 with NaOH, again evaporated to dryness to decompose NH<sub>3</sub>, residue taken up in distilled water and solution evaporated almost to dryness, HCl was added and the solution made up to standard volume.

An aliquot of the clear solution was analyzed by the ninhydrin method for determination of amino acids according to Troll *et al.*, and total proteins were read from the standard curve.

Table I shows the amounts of total proteins from ten different samples collected in the grain mill area and determined by both methods. Results by the two methods are comparable except for sample 7 which shows an amino acid-micro-Kjeldahl ratio of 1.5. The marked variations between the protein concentrations in the different samples depends on many factors including wind direction, sampling location with reference to the source and the grain mill or plant activity at the time of the sample collection.

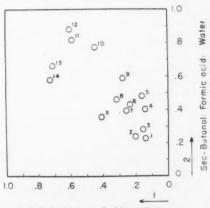
#### Identification of Amino Acids

Experiments were conducted on the airborne particulate samples to prove through amino acid isolation and identification that proteins were present. The sample, after acetone extraction, was hydrolyzed for 20 hours with 6N hydrochloric acid and the solution was centrifuged. The clear supernatant liquid was evaporated to a small volume, separated from the acid and de-

TABLE I

Total Proteins Determined by Micro-Kjeldahl and Amino Acid Methods in Samples of Airborne Particulates Collected in the Grain Mill Area

	Micrograms of	Ratio Amino acid	
Sample No.	by micro- Kjeldahl	by amino acid	micro-Kjeldahl
1	65.5	59.9	.9
2	24.0	25.1	1.0
3	34.7	32.0	.9
4	25.4	29.0	1.1
5	15.8	16.3	1.0
6	5.1	5.2	1.0
7	20.9	31.9	1.5
8	9.6	8.5	.9
9	26.4	27.8	1.1
10	18.0	15.9	.9



Methylathylketone: Pyridine: Water

FIGURE 1. Two-dimensional amino acid paper chromatogram from hydrolyzed material of airborne particulate sample. Solvents: (1) Methylethyl ketone: Pyridine: Water = 70:15:15, (2) Sec. Butanol: Formic acid: Water = 70:15:15. Amino acids identified: 1. Arginine; 2. Histidine; 3. Lysine; 4. Aspartic acid; 5. Glutamic acid; 6. Glycine; 7. Serine; 8. Threonine; 9. Alanine; 10. Valine; 11. Phenylalanine; 12. Leucine; 13. Methionine; 14. Tyrosine and X unidentified spot.

salted by means of an electric desalter according to Consden. A two-dimensional paper chromatogram was run on this material following the method of Turba. 5

Whatman No. 1 filter paper was washed with 0.2% ethylenediaminetetraacetic acid, rinsed with distilled water and dried. This organic acid forms soluble salts with traces of inorganic cat-

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ions in the filter paper and prevents the formation of double or "ghost" spots of amino acids.

Color development of the dry chromatogram was accomplished by dipping into ninhydrin reagent (0.25% ninhydrin in acetone w/v) and heating in an oven at 76°C for 10 minutes. The chromatogram was stored in the dark and read the following day. Fourteen amino acids were identified as shown in Figure 1. The ninhydrin positive spot marked "X" was not identified and may represent an alpha amino acid or a peptide. It is possible that there are trace amounts of other amino acids in the sample, but owing to the limit of sensitivity of the method, they did not appear after color development.

# **Isolation of Specific Proteins**

To isolate proteins from plant cells quantitatively it is necessary to break the cell membranes. In experiments on whole grain, grinding is used to accomplish this. In this work the complete removal of particulate matter from the fibre glass filter is extremely difficult. Extraction of the proteins from the particulate matter in the filter was tried with different solvents namely: 2–10% sodium chloride solution, 0.05N hydrochloric acid, 0.05N sodium hydroxide and 65% ethanol.

Fibre glass filter containing airborne particulates was cut in small squares, placed in 250 ml beaker and cooled by ice in a Dewar flask. One hundred ml of solvent at 0°C were added and the contents mechanically stirred for 8 hours. After centrifuging, the clear supernatant was dialyzed in a cellophane tubing for two hours against distilled water at a temperature near 0°C. The protein solution was concentrated in a vacuum desiccator under conditions of low pressure and 0°C and the proteins separated by electrophoresis. A Reco model E-800-2 electrophoresis apparatus was used in this work with a barbiturate buffer at pH 8.6, ionic strength 0.05.

Proteins exist in aqueous solutions in the form of dipolar ions and will move in an electric field in the direction influenced by the net charge. At the iso-electric point the net charge is zero and no movement takes place. At pH values more acidic than the iso-electric point, the net charge is positive and the proteins move toward the cathode and on the alkaline side the reverse occurs.

Whatman filter paper #3MM was saturated with buffer, blotted dry, and placed in position. The electrolyte vessels were half filled with buffer solution. A small portion of the sample was applied to the previously marked origin on

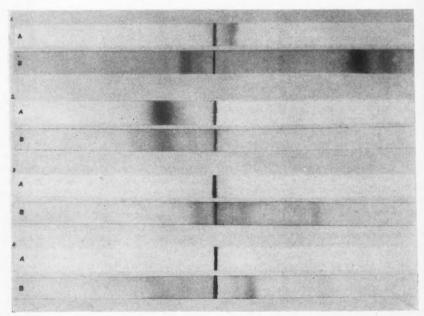


FIGURE 2. Electrophoresegrams of proteins (A) and free amino acids (B) from different solvent extractions of an airborne particulate sample. (1) 10% sodium chloride; (2) 0.05N sodium hydroxide; (3) 0.05N hydrochloric acid; (4) 65% ethanol.

the paper. A potential of 25 volts per centimeter was applied for six hours at room temperature (26-27°C). The electrophoresegrams were dried in a horizontal position at room temperature, then for 10 minutes at 100°C to stabilize and denaturate the proteins. For locating the proteins, the Durrum staining procedure was used.

After staining for 16 hours, the paper was washed twice with 2% acetic acid for five minutes and again with 2% acetic acid for 10 minutes to remove the excess dye. A final rinsing was made for two minutes in 10% acetic acid containing 2% sodium acetate. The rinsing time is very important since the results of photometric scanning may be changed if a different rinse program is used.

Strips from the same electrophoresegrams were developed with 0.25% ninhydrin in acetone to determine the presence of amino acids. In all determinations free amino acids were found to be present in addition to specific proteins. These amino acids may have been split off from proteins in the initial extraction or prior to that in some of the grain processes.

Typical electrophoretic patterns on concen-

trated solvent extracts of an airborne particulate sample collected in the mill area are shown in Figure 2. A comparison of the patterns demonstrates the protein and amino acid solubilities in the different solvents. The 10% sodium chloride solution (1) extracted a protein with a positive charge while 0.05N sodium hydroxide (2) shows negatively charged proteins. Amino acid separations were likewise quite different for the four solvents used. The 0.05N hydrochloric acid and 65% ethanol extracted smaller amounts of positively charged proteins as the markings are quite faint.

Identification of the proteins represented in these electrophoresegrams is being considered. According to Osborne, albumin and globulin are extracted with salt solutions, prolamins in 65–70% ethanol, glutelins in alkali solutions. Recent work using physicochemical methods has shown that these solvents will extract a mixture of many components and not single compounds.

For purposes of comparison, samples of wheat bran collected in a mill near wheat unloading operations were processed through the same protein separation procedure as the area samples.

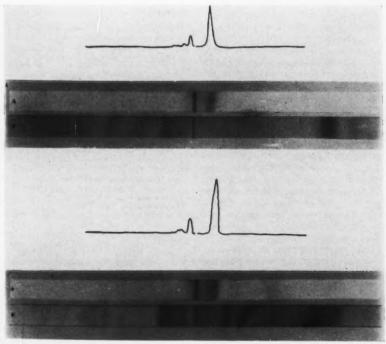


FIGURE 3. Electrophoretic patterns showing proteins (A) and amino acids (B) extracted with 10% sodium chloride: (1) from an area sample of airborne particulates and (2) from wheat bran. Protein concentrations are indicated by the densitometer curve.

Typical electrophoretic patterns of proteins and amino acids in the mill and area samples are shown in Figure 3. The extraction solvent for both samples was 10% sodium chloride. For better comparison each protein strip was scanned in a Photovolt densitometer. The peaks of the curve show the location and quantitative amounts of proteins separated. Similarity of these patterns indicates a possibility that the proteins in airborne particulates from area samples are coming from the grain mills.

#### Discussion

The work reported here is preliminary to a full scale study of the role of proteins in the asthma problem at the University of Minnesota. Further development of total protein determinations and of specific protein separation from airborne samples is in progress.

Accurate determination of total proteins is desired for studying the dispersion of grain dust from the mill and plant area. It has been stated that seed and grain proteins have a higher nitrogen content than animal proteins. Since an animal protein was used as standard for the total protein determinations, the selection of a standard other than egg albumin seems necessary.

In protein separation, reproducible results are obtained only when conditions are carefully controlled. The protein molecule is composed of one or more polypeptide chains to which non-proteins can be attached more or less firmly. The biological, chemical and physical properties of proteins depend upon the integrity of the entire structure. Since a slight change in the structure of the protein molecule may lead to alteration of its properties, it is important to avoid or to control changes in the molecule during the isolation procedure. Changes can occur through chemical reactions, association with other proteins or non-protein materials and dehydration. Presence of reducing substances in the airborne particulate sample has an effect on the solvent extraction.11, 12 Concentration, temperature, and pH of the extracting solvents are important variables that can change results.

The influence of temperature in solvent extraction of the samples, in the concentration of the extracted proteins and in the electrophoretic separation, was noted in this work. Experimentation at higher separation potentials with shorter runs resulted in the loss of proteins and an in-

crease in the amounts of amino acids. In the separation of proteins further work is contemplated on the variables mentioned. The use of a separation medium other than filter paper is also being considered.

#### Summary

Samples of airborne particles collected near grain industry mills and plants have been analyzed for protein amounts and types. After preliminary extraction with acetone to remove the non-protein organic matter, total protein determinations were made by micro-Kjeldahl and amino acid methods. The results on samples collected in the mill area are comparable for both methods. Following hydrolysis of an airborne sample, fourteen amino acids were identified by a two-dimentional chromatogram.

Electrophoretic patterns of proteins separated from other particulate matter were made on several air samples. Similar patterns were obtained on samples of wheat bran collected during an unloading operation in a grain storage plant. Further experimental work is planned on separation and identification of specific proteins.

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# Organization of a Radiation Hazard Emergency Response Capability

A. J. BRESLIN, W. B. HARRIS, and A. A. WEINTRAUB

U. S. Atomic Energy Commission, Health and Safety Laboratory, New York, New York

The U. S. Atomic Energy Commission in cooperation with the Department of Defense maintains a nationwide organization of radiation safety teams to provide emergency technical assistance to state and local officials in the event that an accident with public health significance occurs during the utilization, handling, or transportation of radioactive materials. The capability to handle such accidents is not yet adequately developed at the state and municipal levels. The basic mission of the teams is to provide rapid hazard evaluation and recommendations for control. Important requirements for the satisfactory performance of this mission are (1) effective liaison with civil authorities, (2) expeditious team mobilization and transportation, (3) use of professional radiation safety personnel, (4) field equipment suitable for rapid, approximate measurements.

FROM its earliest days the Atomic Energy Commission has responded to calls from its contractors, other agencies, municipal and state authorities, and private citizens for assistance at accidents involving radiation. Our own laboratory has an extensive history of such calls ranging in seriousness from questions about the importance of radiation emitted from radium-dial wrist watches to an explosion which resulted in injury to several persons. Certainly, the AEC has not been alone in providing this kind of service. Almost every agency and industrial concern having anything to do with the atomic energy program has been called upon from time to time to provide assistance in connection with radiation incidents. Occasionally, these incidents have involved sizeable quantities of radioactive materials and in some cases presented significant radiological hazards, but these have been the exceptions. By and large, the incidents have not been serious although this was generally not knowable until specialists had surveyed the incident and made evaluations.

Early in 1958 the AEC Headquarters formalized this radiological assistance capability in an interim AEC Radiological Assistance Plan. The United States and its possessions were divided into eight geographical regions with an operations office assigned responsibility for organizing and directing the assistance for each region. Responsibility for Region I, which includes the New England States, New York, New Jersey, Pennsylvania, Maryland, Delaware, and the District of Columbia, was assigned to the New York Operations Office. A somewhat similar capability

was established at approximately the same time by the Department of Defense. The organizations of the two agencies are coordinated to effect mutual assistance if required.

In the two years of its existence the Region I organization has responded to approximately thirty calls for assistance, fifteen of which were eventually confirmed to involve radioactivity. As a consequence of its early experiences in providing assistance and the experience of setting up and operating the program for Region I, the New York Operations Office has formed some opinions about the utility of the radiological assistance program, optimum methods of operation, and future developments which are submitted here for your consideration.

One might logically ask at this point what prompted the decision to create this organization. Commonly an emergency group or an emergency plan is developed out of the necessity brought about by a history of events. This was not a cause in the present instance, for although there have been accidents in the atomic energy program, the over-all safety record has been excellent. Since 1943 there has been but a handful of injuries attributable to radiation exposure. Furthermore, these accidents occurred at major facilities with adequate health and safety staffs where the services of an emergency response group were not required. However, it is only reasonable to assume that the accident rate will increase because of the rapid growth of the program. This growth is difficult to quantitate but selecting just one simple index as an indication, the number of radioactive isotopes shipments from Oak Ridge in 1946 was approximately 1000, whereas in 1959 it was approximately 13,000; the 1959 shipments involved more than 200,000 curies. Add to this the isotopes produced and shipped from other laboratories, the processing and utilization of feed materials and special nuclear materials, the manufacture and distribution of weapons, the processing and disposal of radioactive waste materials, and the return of spent fuel for reprocessing and the accident po-

tential may be appreciated.

Where are the services of a radiological assistance team likely to be required? For the purposes of this discussion let us arbitrarily separate those accidents that happen at facilities and those that happen during transport between facilities. As a first approximation, the potential seriousness of an accident is in proportion to the quantity of radioactive material available for involvement. It follows then, that in contrast to any foreseeable transportation incident the potential of an accident at a fixed facility can be greater by many orders of magnitude. Furthermore, frequently present at the fixed facility are the means to release and spread radioactivity such as chemical processes, high temperatures, high pressures, and the like. A reactor may contain all of these elements and consequently is regarded by many as potentially the greatest menace. However, with a few notable exceptions, the effects of accidents that have occurred at fixed facilities have been localized and have been handled by the safety and medical staff of the facility. This is an important distinction between the fixed facility incident and the transportation incident. At any installation where important quantities of radioactive materials are present, specialists are at hand to cope with emergency situations and additional assistance is not ordinarily required. However, in the situation where the public may be affected and where the staff is unable to cope with the consequences of an incident, the radiological assistance program would fill a definite need.

With the exception of escorted shipments, the transportation of radioactive materials presents a quite different situation. Persons familiar with radiological hazard evaluation and safety measures are ordinarily not in attendance. Of course it was this very factor that compelled the framers of the Interstate Commerce Commission shipping regulations to restrict the quantities that may be shipped in a given container and vehicle, and to issue rigid specifications for containers. Indeed, upon examination of the regulations it is difficult to conceive of an accident that could truly result in a radiological injury. Nevertheless, accidents do not conform to specifica-

tions and standards, and exceptions to the ICC regulations are made.

Another important difference between the fixed facility incident and the transportation incident is that the latter almost necessarily must occur in a public or semi-public location. Thus, there are two predisposing conditions to consider in regard to the consequences of a transportation incident. These are (1) the absence of radiation safety specialists, and (2) public accessibility. To these one might also add the psychological factor that is common to most aspects of public involvement with radiation.

Therefore, the situations for which the radiological assistance program was conceived are incidents that create a potential radiological hazard to the public and for which specialized assistance is unavailable or available to an insufficient degree, and problems of great psycho-

logical impact.

Let us examine the manner in which the organization is intended to function. The principal purpose of this program is to provide prompt assistance at any incident involving radioactive material that has potentially serious consequences in terms of injury or property damage. Toward this end, a national network of teams staffed and equipped for radiological hazards evaluation, is maintained. The teams must be able to provide assistance upon call from any location and at any hour. To simplify the procedure there is but one telephone contact in each region, that of the operations office having primary responsibility. For instance, in Region I a telephone call to the New York Operations Office may prompt the dispatch of teams from several locations in addition to New York City, depending on the location of the incident. Of course, for this service to be effective the existence of the program and the telephone number to be contacted must be widely known.

At the time that the program was officially formulated the Commission conducted a rather intensive campaign by letter and personal contact, to notify state and municipal authorities and AEC contractors and licensees about the operation of the program and the means of obtaining service. This campaign has been continuing at a somewhat reduced pace through efforts of the individual operations offices with regional re-

sponsibility.

What, specifically, is the function and what must be the capabilities of the radiological assistance organization? In our opinion the function of an assistance team is to establish the facts regarding the radiological hazard as promptly as possible and make these known to local authorities along with recommendations for the

protection of personnel and property. It is important to recognize that in the general case the Commission team would be operating in an area where it lacks legal jurisdiction. For instance, in a typical transportation accident, civil authorities would have basic responsibility for the protection of individuals and property and the Commission would act solely in an advisory capacity.

In order to fulfill the basic objective cited above, specialists in radiation safety, medicine, and public information must be available to be dispatched to the site of the incident as quickly as possible. This group must be equipped for performing a rapid and reasonably accurate, if not precise, evaluation of the radiological hazard. It must be prepared to cope with a wide variety of circumstances involving an almost unlimited variety of radioactive materials. It must be prepared to estimate the seriousness of the hazard and translate this into terms comprehensible to officials at the incident. It must be in a position to provide specific recommendations for the control of the radiation hazard. It must be able to reply to the questions of representatives of the news media in such a way as to disclose the facts without causing unwarranted alarm.

A key word with regard to team operation is speed. It should not be necessary to elaborate on the importance of this concept. There has been ample evidence of the wild rumors, near panic, and misdirected action that occur in the absence of reliable information in incidents where the public has received some word, usually incomplete or garbled, about a release of radioactivity. The aforementioned explosion incident is a case in point. In the absence of facts rumors develop without limit and the possible consequences of the spread of misinformation could be far graver than the potential hazard from the radiation itself. In fact, if the team were called to an incident at which no or possibly minimal radiation was involved, the simple pronouncement from such an authoritative source to the effect that no hazard existed would more than justify the time spent by the team in its mission. On the other hand, in the situation where the hazard is serious, the importance of speed is self evident.

A second important aspect concerning the radiological assistance organization is that the team membership be restricted to professionals. By professionals we mean persons whose vocation is in the field of health protection or public relations with special emphasis on radiation protection. This remark is perhaps gratuitous with regard to the Commission radiological assistance personnel who are drawn from laboratory and industrial radiation safety staffs, but we have a

special reason for mentioning this and will discuss it more fully later on.

A third important consideration in setting up the assistance organization is the selection of field equipment. On the one hand, it must be adequate to the job but it must not be so bulky or complex that it delays team departure, travel, and evaluation operations at the site. In our opinion, using only a few of the standard field survey instruments, one should be able to estimate acute radiation hazards with fair accuracy. This estimate will enable the team to determine the necessary precautions to minimize immediate damaging effects. If it is determined from the initial survey that more precise and detailed measurements are required, as might be needed in the case of wide-spread contamination, additional personnel and more sophisticated instrumentation can be moved to the site for detailed follow-

Now briefly, we'll describe the New York Operations Office radiological assistance organization and method of operation. Notification to New York Operations Office that an incident has occurred, presumably is by telephone. When a call is received it is immediately referred to a radiation duty officer. His immediate action is to obtain as much information as possible concerning the incident from the caller and to offer recommendations if he believes that the information is sufficiently reliable to enable him to make an interim evaluation. If, in his judgment, the call is in connection with a bona fide incident his next action is to initiate team mobilization. During working hours this is simply a matter of calling within the office approximately a half a dozen individuals from a pool of twenty radiation safety personnel. In addition, a public information officer and a physician may be called. In the non-working hours situation a similar procedure is followed with the exception that individuals must be called, in turn, at their homes. The next step then is team transportation. In the New York Operations Office we are particularly conscious of transportation because of the inherent difficulties of traveling thru and out of a highly congested metropolitan district. This is especially true where team personnel may live as far as 60-75 miles apart. For this reason we have several options of travel procedure depending on the time of the incident and the relative distance from the city. For maximum flexibility field equipment is stored both at the office and in the home of each team member who would be expected to perform a monitoring function. Furthermore, each team member is equipped so that he can operate as an independent monitoring unit. Thus, although a nominal team consists of about five individuals, in special circumstances, particularly if a substantial time-saving could be anticipated, an individual team member could be dispatched to the incident possibly in advance of the main team body. Several methods of transportation have been arranged for. These are private and government vehicles, U. S. Coast Guard helicopters, and U.S. Air Force transport aircraft. To obtain optimum advantage from these various forms of transportation and to cover the various kinds of travel requirements that might confront us, four different team mobilization and travel plans were developed. These depend on the time of the call and the locality of the incident. These are for the working hours local incident, the working hours distant incident, the non-working hours local incident, and the non-working hours distant incident. We do not necessarily advocate the construction of such a complicated travel plan but in our own situation we can think of no feasible alternative.

To provide assistance in mobilizing and transporting the team and to accomplish other vital functions an administrative group is set up at the New York Operations Office at the same time that the team is being notified. This administrative group performs such actions as initiating necessary contact with other agencies, notifying AEC Headquarters, arranging for continuing ground transportation for the team should it be traveling by air, and serving generally as a communications hub.

In addition to the primary team personnel, advance groups consisting of similarly equipped and trained persons have been set up at six locations strategically located throughout the region. The function of these groups is to provide a more rapid response than might be possible with the main team.

Team action at the site of the incident is left to the judgment of a team captain who is responsible for team activities in the field. After careful consideration, when team procedures were originally drawn up, it was decided that operations at the site must be a matter of judgment rather than a manual procedure because of the difficulties in anticipating and prescribing proper action in a manual for the almost un-

limited variety of circumstances that might constitute a radiological incident.

So much for the description of the New York Operations team organization and operation. We would now direct attention to an interval of time not yet covered in our discussion. That interval is between the time that the incident occurs or is discovered, and the arrival of the radiological assistance team. In all probability the first individual with authority to be at the scene would be a policeman or a member of some other kind of emergency agency. What is he to do to prevent injury to bystanders and to protect property from possible damage from the effects of the incident? The AEC has sought to help to meet this contingency by issuing emergency action recommendations to local civil authorities. There are two sets of recommendations, one applying to a weapons incident and the other applying to the more general situation involving radioactivity. In both cases the recommendations pre-suppose no familiarity with radiation hazards or their control. They are general in nature and primarily deal with rescuing injured persons, taking other emergency action, avoiding the immediate effects of radiation. However, if properly followed, these recommendations cover substantially all that need be done in the way of initial action and if the delay pending the arrival of the assistance team is acceptable, no further immediate action need be taken. Suppose, however, the delay is not acceptable such as might be the case where an important road artery is blocked by a radioactive cargo spilled as a result of a trucking accident. This, admittedly, is a gray area. One proposed remedy is that all patrol cars be equipped with radiation survey instruments and that all policemen be trained as radiation safety specialists. This, we think you would agree, isn't feasible but we would also submit that it is unnecessary. The probability that an individual policeman would be called upon to use this training is vanishingly small. Furthermore, there is no more reason to expect all policemen to be radiation safety specialists than there is for them to be expert firefighters, explosives specialists or physicians. But it is important that persons who are immediately responsible for the protection of the public should be able to obtain the specialized assistance when it is needed, whether it concerns radiation or any other hazard not of a routine nature. Insofar as the radiation incident is concerned, certainly the AEC will provide assistance if requested. However, there are already a few states and some large municipalities that are developing radiological assistance capabilities of their own. In New York City, the police laboratory and the Office of Radiation Control are coordinated to provide this service. Massachusetts has conducted extensive training among police and firemen through the efforts of the state police. AEC Headquarters has been conducting training courses upon request for police and firefighting agencies for several years. It is only logical to assume that eventually such capabilities will become available throughout most of those areas where they are most likely to be needed. But here we would like to offer a word of caution about the make-up of local and state organizations with special reference to the need for professional personnel, the topic mentioned earlier in this discussion. In a number of the incidents that the New York Operations Office has handled, individuals and groups have become involved because they had been exposed to a smattering of radiological training. Radiation measurements and other related information obtained from such people have been found to be unreliable in most cases. For instance, more than once we have been told about dose rates of the order of r/hr which turned out to be mr/hr or less when confirming measurements were made. We have also encountered situations where these individuals lacked the fundamental knowledge to check their survey instruments for proper performance. Invariably, they have lacked the experience to judge whether a radiological situation is hazardous or not. This point is stressed because although a tendency to call upon local people for assistance is to be encouraged and commended, it is also obviously important to select local assistance that is truly qualified. It has been our observation that exposure to a short course in radiological training is insufficient to produce persons qualified to handle radiological incidents. However, there are local and state groups that do possess the necessary qualifications and these of course should be called upon for assistance.

To return to the matter of training for the policeman at the local level, we feel that of basic importance is that he be able to recognize that an incident involves radioactive material. In other words, he should be familiar with Interstate Commerce Commission shipping labels and the common radiation warning symbols. Secondly he should be acquainted with the relatively straight-forward procedures that may be followed to minimize injury and damage. Thirdly, he should know that he can obtain specialized assistance. The New York Operations Office takes advantage of every opportunity that occurs to talk to local and state law enforcement agencies and other groups with emergency responsibility in order to spread these concepts within Region I.

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Another partial remedy for this gray area that

exists between the time that an incident is reported and the time that an assistance team arrives, is to attempt to develop a partial evaluation of the incident and furnish interim recommendations by telephone. This procedure of course has its pitfalls. For instance, we have found that the information given by a private citizen is frequently garbled and subjective. But policemen and other persons who are trained to be objective can and do furnish completely reliable information that although non-technical in nature, can be used to evaluate the accident. Conceivably, recommendations issued on the basis of limited information of this nature could be of great value pending the arrival of the team.

# Summary

In our view the Commission is filling a void of local radiological capability that now exists quite generally throughout the country. The New York Operations Office with responsibility for the northeast part of the country has established a network of teams that can be dispatched at a moment's notice to any locality within Region I. The teams are highly mobile and equipped with the minimum instrumentation consistent with accurate hazard evaluation capability so that travel and evaluation at the site can be rapid, and factual information can be presented to local authorities in the shortest possible time. To best fit the needs of this present system there are two areas of particular importance that warrant further emphasis: (1) the existence of the AEC capability and the means of contact must be widely known, and (2) members of local and state law enforcement agencies and other emergency agencies should be able to recognize shipments of radioactive cargo and have familiarity with emergency precautionary procedures. Full use should be made of local and state radiological capabilities for emergencies but in this regard it is important to select only qualified persons for this service. It is anticipated that eventually all of the states and the larger municipalities will develop their own radiological assistance capabilities within appropriate bureaus such as health departments, labor departments, police agencies and others. At that time the urgent requirements for a group such as we have described will decrease.

# The Determination of Airborne Radioactivity

L. R. SETTER, Ph.D., and G. I. COATS, B.S.

Division of Radiological Health, Robert A. Taft Sanitary Engineering Center, U. S. Public Health Service, Cincinnati 26, Ohio

Practical procedures for the gross differentiation and quantitative determination of natural and artificial airborne particulate radioactivities are presented. The proper choice of instruments and time of counting is necessary for the separate determinations. Details of methods are given with formulae for calculations.

#### Introduction

AIRBORNE particulate radioactivity may be of natural occurrence such as, for example, the daughter products of radon or thoron which emit alpha, beta and gamma radiation. It may also be artificial radionuclides formed by nuclear reactions. The artificial radionuclides usually emit beta and beta-gamma radiation. In general, detailed differentiation of natural and artificial radionuclides is difficult. This paper presents practical procedures for the gross differentiation and quantitative determination of natural and artificial airborne particulate radioactivities.

#### **Natural Radioactivity**

The composition of the earth's crust includes traces of uranium and thorium compounds, both of which decay through a series of daughter elements. Depending on meteorological and soil conditions (pressure, temperature, permeability, moisture, etc.), the gaseous radon and thoron may diffuse into the atmosphere prior to decay to daughter products which are solids. Since only the alpha activity is of interest, the calculations can be simplified by assuming the following decay schemes:1

$$\begin{array}{c} \operatorname{Rn} \xrightarrow{\alpha} \operatorname{Ra} \operatorname{A} \xrightarrow{\alpha} \operatorname{Ra} \operatorname{B} \xrightarrow{\beta} \operatorname{Pb}^{214} \\ & \operatorname{Ra} \operatorname{C} \\ & \operatorname{Ra} \operatorname{C} \xrightarrow{19.7 \mathrm{m}} \operatorname{Ra} \operatorname{D} \\ & \operatorname{Pb}^{216} \end{array} \xrightarrow{\alpha} \operatorname{Ra} \operatorname{D} \\ & \operatorname{Tn} \xrightarrow{\alpha} \operatorname{Th} \operatorname{A} \xrightarrow{\alpha} \operatorname{Th} \operatorname{B} \xrightarrow{\beta} \operatorname{10.6 \mathrm{h}} \\ & \operatorname{Pb}^{212} \xrightarrow{\alpha} \operatorname{Th} \operatorname{C} \xrightarrow{\alpha} \operatorname{Ch} \operatorname{Bi}^{212} \end{array}$$

Presented at the Twenty-first Annual Meeting of the American Industrial Hygiene Association, Rochester, New York, April 28, 1960.

During the filtration of air through a membrane filter the radon and thoron daughter products are deposited on the filter. The alpha radioactivity of the filter (Ra A, Ra C, and Th C) can be theoretically related to the concentration of radon or thoron in the air.1, 2,

Radon daughters have a composite alpha halflife of approximately 0.5 hour and the thoron daughters have an alpha half-life of 10.6 hours. If the gaseous activity of the air remains constant, the alpha activity of the filter will reach 99.6 per cent of equilibrium in four hours for radon daughters and in 84 hours for thoron daughters, as indicated in Figure 1. Equilibrium exists when the decay rate equals the rate of deposition on the filter for a given concentration of parent gas.

In practice, the sample collection period may be less than that required for equilibrium, as corrections for non-equilibrium can be made. Figures 2 and 3 show correction factors applicable for various sampling times and for delayed counting. When sampling for radon only, a short sampling period of a few hours is desirable, as the small interference from thoron

activity may be neglected.

Usually there is much less thoron present in the air than radon. This is probably due to the shorter half-life of thoron (54.5 sec) as compared to radon (3.8 days), since the latter can be airborne from greater depths of material. When the sampling period is long or the thoron activity is high, the thoron contribution may be a significant fraction of the total alpha activity. Thoron daughter products may be differentiated from radon daughter products by counting the alpha activity after the decay of the shorter lived radon daughters. Thoron daughter product activity may then be extrapolated by means of Figure 3 to the time of sample collection. Subtraction of the thoron daughter activity from the total alpha activity at the time of collection

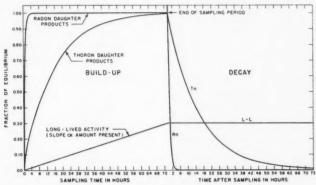


FIGURE 1. Alpha activity on filter paper.

gives the radon daughter alpha activity. Thus, both the radon and thoron activity concentrations may be calculated.

For longer-lived activity such as long-lived alpha or the beta radiation of fission products (bomb debris), the activity in the air is more nearly a function of the total volume of air sampled rather than the sampling rate. The long-lived alpha activity is measured by counting the filter after the thoron activity has decayed. Airborne nuclear bomb debris particulates usually have no more than traces of long-lived alpha activity.

# Apparatus: (See Figure 4)

- (a) Vacuum pump 1-2 cfm capacity (self-lubricating Gast or equivalent).
- (b) In-line membrane filter holder (Millipore XX5004700, Gelman SH2, or equivalent).
- (c) Membrane filter with a pore size of 0.8 microns such as Millipore type AA, Gelman type AM4, or equivalent.
- (d) Air flow meter having a range of 10-40 liters per minute. This may be a rotameter or the air flow may be calibrated in terms of a differential manometer reading across the filter. If the rotameter is operated in the line, corrections must be made for the pressure drop in the line from the filter to pump. Additional corrections may be made for changes in temperature and atmospheric pressure although these are usually considered minor enough to be neglected.
- (e) Alpha scintillation counter (zinc sulfide screen) complete with photomultiplier tube, scaler, cathode follower, high voltage supply, timer, and register. An internal proportional counter could also be used

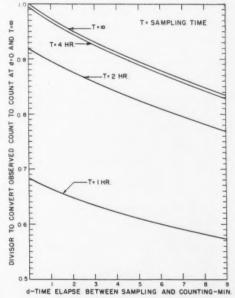


FIGURE 2. Conversion chart for radon daughter products.

## Procedure:

- (a) At the time of sample removal from the filter holder, record the sampling rate, length of sampling period, and the time of sample removal.
- (b) The sample is alpha counted on the scintillation counter as soon as possible. The time from the end of sampling period to midpoint of counting is recorded. For greater accuracy in long counts the "exponential center" of the counting period

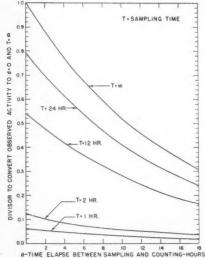


FIGURE 3. Conversion chart for thoron daughter products.

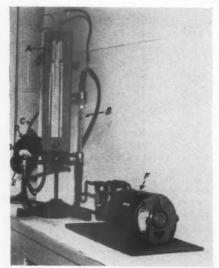


FIGURE 4. Air sampling system: A—manometer, B—flow meter, C—inlet for outside air, D—membrane filter holder, E—membrane filter, and F—air pump.

or 31.6 per cent of the counting time can be used. A counting period of sufficient length to reduce the counting error to a reasonable value should be used. To keep the standard deviation less than five per cent (10 per cent for the 95 per cent confidence level) the following table can be used as a counting guide.

Counting Time for a Standard Deviation of 5 Per Cent

Counts/minute	Counting Time (min		
400	1		
200	2		
100	4		
50	8		
40	10		

- (c) The sample is recounted after the decay of the radon daughter products. A decay period of eight half-lives or four hours will reduce the radon daughter interference to less than four per cent (assuming that the thoron daughter activity is 10 per cent of the radon daughter). The time from sample removal to this counting period is recorded.
- (d) If the thoron daughter contribution is neglected, a filter may be removed from the sampling head, counted, and immediately replaced. (In the Public Health Service sampling program the filter is changed and counted at 0800 hours. The new filter is removed, counted, and replaced at 1500 hours. The decay count for the morning filter is then taken.) Also, the filter can be continuously monitored with a detector, ratemeter, and strip chart recorder. The thoron activity is usually found to be a fraction of the radon activity and, for short sampling periods or as a first approximation, may be neglected.
- (e) The long-lived alpha activity is determined by counting after the decay of the thoron daughters. Due to the long half-life, the activity must be calculated on the basis of the volume of air sampled rather than the sampling rate.
- (f) The fission product activity can be determined from the membrane filter in the same manner by beta counting. If the membrane filters are counted in an internal proportional counter, it may be necessary to treat the filter to eliminate difficulties due to electrostatic effects. This treatment may consist of ashing or the application of an anti-static material.

# Radon and Thoron Calculation:

Assuming that the daughter products are in equilibrium, the alpha activities of radon daughter products and thoron daughter products are, according to Mercer<sup>1</sup>;

$$\begin{split} A_r &= RnF \ [4.50 \ (1 \ - \ e^{-0.2274t}) \ e^{-0.2274t} \\ &+ 164.96 \ (1 \ - \ e^{-0.0258t}) \ e^{-0.0258d} \\ &- 95.67 \ (1 \ - \ e^{-0.035t}) \ e^{-0.035d} ] \end{split} \tag{1}$$

and

$$A_t = TnF [1012.21 \ (1 - e^{-0.0011t}) \ e^{-0.0011d}$$
  
- 9.91 \ (1 - e^{-0.011t}) \ e^{-0.011d}] \ (2)

where

n

Rn = the radon concentration/liter of air Tn = the thoron concentration/liter of air F = sampling rate in liters/minute at end

of sampling period

t = sampling time in minutes, and

d = the time between end of sampling period and time of counting, in minutes.

For an infinite sampling period  $(t = \infty)$  and no decay (d = 0), these equations become:

$$A_r = 73.79 \ F \ Rn$$
 (3)

$$A_t = 1002.3 \ F \ Tn$$
 (4)

When the radon is reported in micromicrocurie per cubic meter  $(\mu\mu c/m^3)$ 

$$Rn = 6 A_{\tau}/F, \tag{5}$$

or according to Harley<sup>2, 3</sup>

$$Rn = 5.95 A_r/F. (5a)$$

Similarly when the thoron is reported in micromicrocuries per cubic meter,<sup>3</sup>

$$Tn = 0.45 A_t/F \tag{6}$$

A calibration of the efficiency of the alpha counting instrument is made to convert observed counts per minute to disintegrations per minute. (Normal efficiencies for thin alpha sources in internal proportional and (ZnS) scintillation counters are 50–51 and 30–45 per cent, respectively.) The observed activity in dpm is corrected for decay from the time of sampling to time of counting and for non-equilibrium sampling conditions (Equations 1 and 2 as presented in Figures 2 and 3). When standard sampling and counting procedures have been established, the factors of equilibrium, decay, and counting efficiency may be combined with the constant in Equations 5 and 6.

In all calculations the membrane filter is assumed to have a collection efficiency of 100 per cent and no self-absorption. This is in view of the collection efficiency for Millipore type AA and HA of 99.9° per cent as reported by Smith and Surpenant, and an alpha self-absorption of three per cent for radon daughter products on Millipore filters as measured by Shapiro. This three per cent self-absorption is considered insignificant in view of the errors in air flow measurement, counting statistics, and non-equilibrium conditions.

The scintillation counter is calibrated by comparing its counting rate on an air sample with an internal proportional counter. A radium D and E standard is used to determine voltage plateaus and to check instrument performance.

#### **Artificial Radioactivity**

Airborne fission product activity can be monitored by deposition on filters with subsequent analysis by gross beta counting. These gross beta values give information on fluctuations in the existing levels. Also, for a single test blast, an estimate of the age of the activity can be obtained by the decay rate.

## Apparatus:

The apparatus described below is that used in the Public Health Service Air Sampling Network. Other equally effective systems could be used.<sup>6</sup>

- (a) High volume air sampler, with calibrated flow indicator, (40-60 cfm) designed to accept an 8 x 10 inch filter.<sup>7</sup>
- (b) Glass fiber filter 8 x 10 inches in area (MSA-1106B or equivalent). The dust particles are deposited on a 7 x 9 inch area.
- (c) Large area (7.5 inches diam.) proportional gas-flow counting chamber and preamplifier (NMC Model PCC-12A or equivalent) converted to a thin mylar window detector, and connected to a conventional scaler. The filter sample, placed on a sliding tray, is centered under the chamber 0.5 cm below the mylar window.
- (d) Analytical balance to measure the total solids on the filters.

#### Procedure:

- (a) The sample is routinely taken for 24 hours. The average air flow is determined from the initial and final readings.
- (b) The amount of dust collected on the filter is determined by weighing the filter

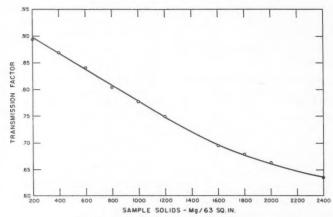


FIGURE 5. Beta transmission factor.

before and after sampling. The filters and filter samples are equilibrated in an incubator at 70°F and 50 per cent humidity for 16 hours before weighing.

- (c) The first count is taken 3-4 days after sampling to allow for the decay of natural activity. A second count for decay information is taken four to five days later.
- (d) Additional decay counts can be taken if desired. The shorter half-life or "fresher material" will require more decay counts.

#### Calculations:

The filter counting rate is extrapolated back to the midpoint of sample collection by plotting the decay rate on graph paper. If the rate of decay is relatively low, indicating fission products formed at least 10 days before the first count, the sample counting rates are plotted on semi-log paper and extrapolated back to the time of sample collection.

If a rapid decay rate is observed, the counting rates are plotted on log-log paper, (with the age of the material as the abcissa), in such a fashion that the Hunter and Ballou<sup>8</sup> exponential decay factor of -1.2 is obtained, i.e. the slope of the curve (dy/dx) is -1.2. A more accurate extrapolation is performed if more than one decay count is made. This method of plotting also gives an estimate of the age of the material.

The counting rate is then converted to  $\mu\mu c/m^a$  according to

$$\mu\mu c/m^3 = \frac{cpm}{(2.22) \text{ (counting efficiency) (vol. of air)}}$$

The counting efficiency includes the absorption, geometry, and backscatter. Allowing for

filter overlap, and radial collection efficiency of the chamber, 66.5 per cent of the filter area is counted. The counter efficiency was determined to be 56.3 per cent using a thallium-204 source. The collection efficiency of the filter is assumed to be 100 per cent.\*

The correction for sample self-absorption is given in Figure 5 as a function of total sample solids on the filter. This transmission factor was derived from an average of 5-day, 1 year, and 2.5 year old fission products, and includes the absorption in the glass fiber filter.

Thus.

$$\mu\mu c/m^3 = \frac{\text{cpm}}{(2.22) (0.563) (0.665) VT} = \frac{1.49 \text{ cpm}}{VT}$$

where V is the total air volume in cubic meters and T is the transmission factor from Figure 5.

#### Summary

Natural and artificial airborne particulate radioactivities are separately determined by choice of instrument and time of counting. The natural activities, presumed due largely to radon and thoron daughters, are counted in an alpha scintillation counter before and after the decay of the radon daughters. The artificial radioactivity is counted for beta-gamma activity after most of the natural activity has decayed.

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# COMPUTER FOR AUTOMATIC WEATHER STATION

THE NATIONAL BUREAU OF STANDARDS in cooperation with the U. S. Weather Bureau has developed a specialized digital computer to be used as a research tool in exploring the possibility of an automatic weather station. This computer is called AMOS IV and receives data from weather sensing instruments and processes these data through such functions as sampling, comparing, selecting a maximum, and arithmetic operations. The results are transmitted via teletype to a central forecasting station and to other airport weather stations.

The automatic weather station is built around a single, small general purpose computer designed especially for this application. The computer receives information from the imput instruments at any desired interval. These data are suitably processed and arranged in a specified order for teletype transmission in a variety of message formats and at various speeds. The computer continuously monitors new imput data while processing data already entered and transmitting messages on command. Data is stored on a magnetic drum which provides for simultaneous read-in and read-out of data by use of dual-head channels. The magnetic drum provides the extensive storage capacity required for the table look-up involved in calculations. While designed primarily for weather use in connection with airports, the automatic weather station would have many other applications and would be especially useful in relatively inaccessible locations that are important sources of early data on meteorological activity.

## TRANSFERRING LIQUIFIED GASES BY PIPELINE

MODERN TECHNOLOGY HAS INCREASED the importance of handling and transferring large quantities of liquified gases such as hydrogen, oxygen, nitrogen, helium, and others. These gases are used as refrigerants and as essential parts in the propellant systems of rockets, missiles, and satellites. In general, the low temperature liquids are transported from the liquifier to the experimental set-up in insulated Dewar-type containers. The National Bureau of Standards has recently undertaken a study which indicates that such liquified gases can be transferred safely, economically, and in quantity by means of insulated pipelines. On the basis of a mathematical model the Bureau has concluded that such a transferring system is possible and is undertaking further studies on the economic and feasibility questions. They are now in the process of experimentally verifying the theoretical conclusions on long-distance transfer systems. An initial report of this work, National Bureau of Standards Circular No. 596 (Dec. 1, 1958), is available from the Superintendent of Documents, U. S. Government Printing Office, Washington, D. C., for 30 cents per copy.

# Communication Media Employed in Health Physics at the Knolls Atomic Power Laboratory

L. J. CHERUBIN, B.S., Ch.E.

Knolls Atomic Power Laboratory, General Electric Company, Schenectady, New York

The health physicist as well as the industrial hygienist and safety engineer have a need for maintaining adequate and effective communications with management, employees and other interested special groups in order to obtain understanding of their effort and objectives and thereby obtaining from those sources their support and acceptance. Communications media employed at KAPL to obtain support and acceptance of the radiation protection activity are discussed in this article with the hope that the interested reader will first consider and evaluate the role of communications as a tool in his own program, and secondly through their membership in various national organizations encourage those organizations to provide them with communication tools that are needed.

#### Introduction

COMEONE—jokingly, perhaps—has said that the formula for success is: "Early to bed, early to rise; work like Hell, and advertise." Industrial hygienists and health physicists are familiar enough with the first three, but I think we tend to shy away-unnecessarily and often to our disadvantage-from "advertising" or telling our public about the results of our work. Seriously, I say from experience that the effectiveness of a radiation protection activity can be improved through a well planned and executed communication program. This paper is concerned with the scope, contents and some details of the communication methods employed at the Knolls Atomic Power Laboratory (KAPL) to "advertise" and therefore enhance the effectiveness of the radiation protection effort.

Why should health physicists be aware of and discharge their responsibilities in the communications area? Well, radiation hazards and radiation control measures are relatively new on the industrial scene. They also have some unique health aspects, for example: the genetic implications as well as the aging influence. In addition the publicity given to the fallout problem has focused attention on radiation hazard consideration in the use of radiation in medicine as well as industry. Since management must justify in its own mind, first, what total manpower and

finances must be allocated to the radiation protection program and, second, what policy should be established in radiation protection matters; management must have an understanding of radiation hazards and programs designed to meet these hazards. And since in one way or another each employee generally is involved in radiation protection considerations, a thorough understanding of radiation as a health problem is desired of each employee. We cannot stop there, however, since through releases of industrial wastes into the environment, the interests of the public and specific governmental agencies are involved as well. Therefore, the radiation protection communication program should encompass the plant membership, the community, and interested and responsible governmental repre-

The remarks that follow will indicate what means have been used at KAPL to "spread the word so to speak" about radiation protection matters.

#### Types of Communications

As would be expected, oral, written and visual aid media of communication are being employed singly or in combination.

Oral communications include safety meetings, educational courses, specific lectures, presentations at staff meetings, and general and individual orientation meetings. These are most valuable because they stimulate questions—and, face to face, the question-and-answer method is the

Presented at the Twenty-First Annual Meeting of the American Industrial Hygiene Association at Rochester, New York, April, 1960.

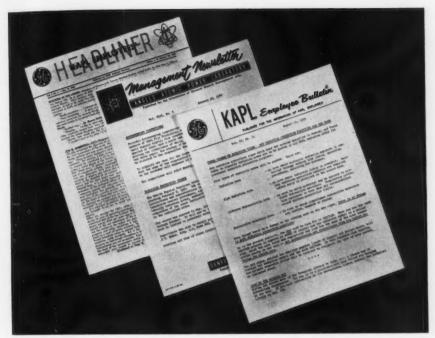


Figure 1. Illustration of the Employee Bulletin, Management Newsletter and KAPL Headliner.

best way of replacing ignorance with knowledge.

Written communications include internal policy guides and procedures; Management Newsletters, Employee Bulletins, and KAPL Headliner (Laboratory paper); local newspapers; the KAPL Radiation Protection Primer; formal reports and manuals. A Reactor Safety Trends Newsletter and the Health Physics Brief are issued periodically as well. (See Figures 1–2.)

Visual aid communications include open house, lobby displays, signs and posters (see Figure 3), and movies.

Special approaches have included a contest conducted within our Laboratory and pictorial newspaper stories in the local press. A slide commentary kit has also been prepared for orientation meetings.

## What Is Communicated?

 Adoption of new radiation protection policies and practices or significant revisions in existing policies and practices, for example, radiation exposure limits, personnel monitoring requirements, radiation area classification and posting, etc.

- Reporting on the radiation exposure trends in the plant and plant environs.
- Orientation of all new employees with respect to radiation and its control.
- Alerting supervisors and employees when we find unfavorable habits developing, e.g., violations of area regulations, use of abrasives or harmful chemicals for skin decontaminations, etc.
- 5. Providing timely information to groups of employees concerned with radiation hazards and their control, since in the course of time questions arise in some employees' minds as to safety, e.g., why we no longer wear film badges in this shop?; or why does the time limit on exposure change from day to day on apparently the same job and in the same area?; or should medical exposures be reported?; etc.
- Trends and developments in radiation protection matters as they relate to reactors, legislative requirements, Atomic Energy Commission standards, company activities, and activities of national and international authorities on radiation protection matters.

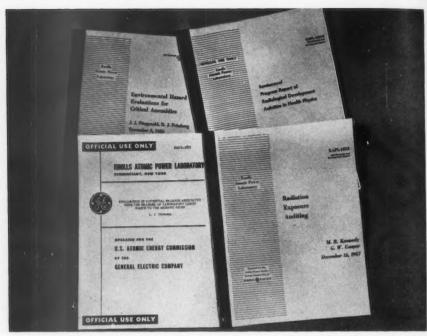


FIGURE 2. Health Physics Topical Reports: Radiation Exposure Auditing, Semiannual Progress Report of Radiological Development Activities in Health Physics, Environmental Hazard Evaluation for Critical Assemblies, and Evaluation of Potential Hazards Associated with the Release of Laboratory Liquid Waste to the Mohawk River.

#### Reaching the Employee

Most of the communications, of course, are directed towards reaching the employees because essentially the potential radiation hazard involves them most directly both in extent and degree. Consequently the whole gamut of written, oral and visual aids communications are employed. Signs and posters, employee bulletins, policy guides and procedures, the plant newspaper, plant lobby displays, safety meetings, and movies are used in varying combinations to put across our point with emphasis, recognizing that repetition is often essential. When you communicate in print, of course, you have to beat your brow hard to be sure you use words that mean the same thing to your audience that they do to you. Little words can mean a lot. You have no idea (or maybe you do) how some of our new employees react when they hear that our Laboratory has a whole group of people who deal with Health Physics.

I would like to discuss sone of our more un-

usual communication efforts, namely, the contest approach.

Several years ago we reached a point where considerable concern arose in our minds about the radiation protection awareness, in other words, the radiation safety consciousness of our employees. Aside from evidence of minor infractions of safety regulations, there was a significant change in job assignments as a result of application of seniority rights. This resulted in an increase of personnel assigned to radiation work with little or no experience. To put the spotlight on the subject of radiation safety, a contest was devised which consisted of:

- Placing various posters, specially designed for the purpose, throughout the Laboratory with cartoons which depicted a worker involved with the application of some basic element of radiation protection.
- Requesting the submittal of a suitable name to characterize the cartooned safe employee depicted in the poster scene.

- 3. Selection of a name winner.
- 4. Award of a financial prize.

You may be interested to know that many individuals participated submitting 479 names. (No. 221, "Ray Alert," was the name selected.) The criteria for selection were:

- 1. A catchy, interesting name.
- A name related to radiation protection, indicating respect for radiation but not fear.
- 3. A name suitable to the poster character.
- 4. A unique but sensible name.
- A name portraying radiation safety consciousness.

Some pertinent and non-pertinent names submitted were: Reddi Eddie, Radiac Joe, Careful Clarence, Danger Dodging Dan, Nodose Dennis, and Mr. Safety T. Wise. Just about everybody got into the act in this contest period. From the management, which supported the contest and made the financial prize award, down through the communications and graphics personnel to all classes of employees, including the PhD level. Incidentally, we have used the Ray Alert character in posters, in our Primer, and in other printed media continually since the contest.

# Reaching the Community

We try to reach the community primarily through local newspaper stories. The open house type visit has been confined to select groups such as employee's family, high school science students, and area physicians. Occasionally, lectures are given to Civil Defense-oriented groups, but this audience is a select one.

### Reaching Governmental Agencies

Governmental groups are reached through formal arrangements such as the one we have with the Mohawk River Advisory Committee which consists of New York State Health Department representatives (medical and sanitary engineers) and New York State Water Pollution Control Board representatives. This committee is briefed of new developments in waste disposal practices at the Laboratory via meetings and field visits and of current environmental effects of liquid waste disposal to surface stream through periodic written reports. The Atomic Energy Commission is, of course, kept abreast of radiation protection programs and their effectiveness through written reports-issued weekly, monthly and quarterly.

#### Communication Skills

We at the Knolls Atomic Power Laboratory attempt to marshall all the expert assistance to

# RAY ALERT Sez: FOR YOUR PROTECTION USE THE BETA-GAMMA FRISKER



FIGURE 3. A health physics poster portraying

a cartoon of Ray Alert using a Frisker

improve our communication effectiveness. Available at the Laboratory are technical writers, graphics skills, editorial writers, and communication specialists. As far as we know, we have not slighted any skill or any media that could reasonably be available to us for communication purposes. Undoubtedly your organization has people who enjoy writing or drawing and, if you're really persuasive, you can entice them to help you with your communication efforts.

#### Communication Needs

The need for transmission of information on radiation protection matters can be generally summarized as follows:

- to transmit knowledge concerning radiation hazards and control measures,
- to alert to potential radiation hazards in the plant,
- to awaken and maintain a radiation hazards awareness among all employees and
- to reassure the employee, management, community and interested governmental groups that radiation hazards are adequately controlled.

What aids do we need to make it easier to spread information about radiation protection? Well, among those most welcome would be

posters, pamphlets and movies on general and selected topics. It appears to me that such organizations as the National Safety Council, the Atomic Industrial Forum, the U. S. Public Health Service, State Health and Labor Departments, the American Medical Association, the Industrial Hygiene Society, the Health Physics Society and insurance companies would be rendering a great and important service if they would take upon themselves the chore and responsibility to make available posters, pamphlets, movies, etc., on radiation protection sub-

jects. Ask yourself, "Am I in a position to help bring this need to their attention?"

The purpose of this paper has been to bring to your attention, as industrial hygienists or health physicists, your responsibilities for applying communications techniques effectively in the discharge of your responsibilities. I believe that, as you develop an awareness of communication needs, you will see ways to encourage your friends and associates in the organizations mentioned above to extend their resources and knowledge to cover the broad communication of information relating to radiation hazards.

# INDUSTRIAL EXPLOSIVES

MORE INDUSTRIAL EXPLOSIVES were used in the United States during 1959 than in any similar period on record, according to the U. S. Bureau of Mines. Domestic consumption of industrial explosives increased nine per cent over the previous year and one per cent over the previous high in 1957. The greatest percentage increase in 1959 was recorded for seismographic exploration with 20 per cent more explosives used than in 1958. Other industries reporting increases were: coal mining, metal mining, quarry and nonmetal mining, and railroad and other construction purposes. A detailed report of the national consumption of industrial explosives is contained in the U. S. Bureau of Mines Mineral Market Report No. MMS 3170.

#### FLAME-SPRAYING OF ALUMINA

THE BASIC PRINCIPLES involved in the flame-spray process of coating metals with alumina are being investigated by the National Bureau of Standards for the Air Force. D. G. Moore, of the Bureau's ceramic coating laboratory, is directing the investigation. Potential uses of the heat resistant coating produced by this process include the thermal protection of jet and rocket engine components, and regulation of temperature within space vehicles. One of the determinants of coating structure is particle velocity; to study this variable, a rotating-disk velocimeter was developed.

Of the three current methods for applying coatings to metal by particle impact—namely, flame-spray, detonation, and plasma-jet—the flame-spray technique offers the advantages of mobility and relative ease of operation. Although the coating of parts by flame-spraying has been practiced for many years, the application of alumina by this means is a relatively new development.

In flame-spraying alumina, two types of oxy-acetylene guns known as the "powder gun" and the "rod gun", are used to melt and propel the particles. In the powder gun, finely divided alumina is fed into the combustion zone, producing a continuous stream of particles. The rod gun is fed by a ½-inch rod of sintered alumina, and air is introduced at the exit nozzle to increase particle acceleration. It was observed that the rod gun normally produces bursts of particles, rather than a continuous stream. The strength of the bond formed between alumina and iron increases exponentially with roughness of substrate surface. The rod gun produces a stronger bond.

# Automatic Sampling and Determination of Micro-quantities of Mercury Vapor

WESLEY C. L. HEMEON and GEORGE F. HAINES, JR.\*

Hemeon Associates, Pittsburgh, Pa.

The AISI smoke sampler supplied with paper impregnated with potassium iodide reagent is employed to sample for mercury vapor in air. Then the mercury is volatilized and is measured by means of a mercury vapor detector. Details of procedure and calibration are given.

A FEW years ago we were engaged in a research effort devoted to the development of a technique for tracing the path of stack gases containing air pollutants over extended distances, work which was summarized in a paper describing the use of antimony oxide powder as the tracer, the AISI smoke sampler for pick-up of this powdered tracer, and subsequent analysis for determination of tracer quantities by neutron activation.

Prior to the work with antimony oxide, we had conceived the idea that mercury vapor might suit these purposes and being gaseous, have features superior to those employing particulate matter. It became apparent, however, that the sensitivity of the best analytical techniques we could devise was insufficient for these purposes. A complete technique was, however, developed, and it subsequently appeared that it would be admirably adapted to industrial hygiene problems where mercury vapor monitoring is desirable.

#### General Method

The AISI smoke sampler is provided with specially prepared filter paper, impregnated with an iodine-potassium iodide solution which has high efficiency for the absorption and retention of mercury vapors. It is not necessary as in the case of the hydrogen sulfide form of this apparatus, to provide a pre-filter for the removal of smoke. A humidifying chamber, however, preceding the filter paper is required.

Determination of mercury is effected by immersing in water an individual section of exposed filter paper which comprises the sample, together with a small piece of polished copper foil, in a 3-inch test tube. Natural deposition of mercury onto copper foil is allowed to proceed

for a period of time. The mercury content of the copper foil is then evaluated by vaporizing the mercury into a stream of air which is then passed through a mercury vapor detector apparatus. A quantity of mercury as small as 0.25 microgram can be evaluated quantitatively with low error.

This technique would probably not have practical application in a situation where determination of mercury vapor concentrations at a single location would be adequate for a complete description of mercury vapor concentrations. Obviously in such a case the vapor detector could be employed for a direct measurement and recording of mercury vapor concentrations. On the other hand, where vapor concentrations are highly variable as between numerous locations, the method permits considerable economy in that only a single vapor detector is necessary.

#### **Procedures**

Preparation of Paper

A satisfactory impregnant consists of a solution of iodine in potassium iodide. Various formulations were made up, applied to Whatman No. 4 filter paper, and the absorption efficiency of each was determined. The results are shown in the Table I.

The efficiency of the impregnated filter paper was found to be markedly reduced in conditions of low humidity. Several tests showed that in a dry atmosphere absorption efficiency is reduced from 90 per cent to as low as 25 per cent. To avoid this, a simple humidifier was included in the sampling line ahead of the filter paper. With this arrangement and using the optimum impregnating formula, numerous tests showed efficiencies exceeding 90 per cent.

#### Optimum Formulation

The impregnating solution formulation finally selected was 4.6% I<sub>2</sub> in 55% KI solution. This is

<sup>\*</sup> Present address: Bethlehem Steel Company, Bethlehem, Pennsylvania.

TABLE I

Absorption Efficiency of Impregnated Filter Papers for Mercury Vapor

Impregnating solution (percentages by weight)	Efficiency %
0.2% I <sub>2</sub> in 2.3% KI	0
0.4% I2 in 4.6% KI	9
1% Is in 11% KI	13
2.5% I2 in 30% KI	33
4% I2 in 48% KI	92

prepared by dissolving 180 gm potassium iodide in 130 ml water, adding 15 gm iodine and diluting to 200 ml. Five milliliters of diethylene glycol is included as a humectant.

# Impregnating Procedure

The impregnating procedure consists of rolling together lengths of Whatman No. 4 roll filter paper and 2-inch gauze bandage and soaking in the impregnating solution overnight. Drying is carried out with a conveyor type drier, the gauze providing strength for handling the wet paper until it has been dried. (The impregnated paper is available commercially from Research Appliance Co., Allison Park, Pa.)

#### Analysis of Filter Paper Sample

# Mercury Vapor Detector

Evaluation of the quantity of mercury trapped by the treated filter paper during the sampling process is effected by a procedure which releases the mercury as vapor into a stream of air which passes through a sensitive mercury vapor detector. We employed the General Electric Vapor Detector. It is sensitive to mercury vapor concentrations down to 0.005 ppm.

Following an unsuccessful series of attempts to volatilize mercury directly from the filter paper into the detector air stream, the method described below was evolved. This method eliminates interferences, reduces high background and gives reproducible results.

The filter paper spot is cut from the exposed roll and placed in a 3-inch test tube. Dilute so-dium sulfite solution (1% Na<sub>s</sub>SO<sub>3</sub> by weight) is then added in sufficient amount to reduce the free iodine to the iodide as indicated by a color change from dark brown to colorless. A piece of brightened copper foil, ¾ square inch in area, is then dropped into the solution and natural deposition of the mercury ions on the copper foil is allowed to take place.

After 24 to 72 hours (see later discussion) the

foil is removed from the solution with tweezers and immediately rinsed successively with two portions of water, two of acetone and one of ether. The washing procedure must consume no more than a few seconds to avoid loss of mercury due to vaporization. The specimen is immediately placed on the heater in the vaporization chamber as shown in Figure 1. With the detector in operation and the air flow controlled at seven liters per minute, the heater is turned on and the intensity adjusted so that the mercury is vaporized within a suitable time period which generally ranges between two and four minutes. The concentration of mercury existing in the detector chamber is continuously recorded on the chart recorder. Typical detector response curves for various quantities of mercury are shown in Figure 2. The area under the curve is determined by a planimeter and the quantity of mercury estimated by reference to a calibration curve.

# Magnitude of Background

The ultimate sensitivity of this analytical procedure is largely dependent upon the magnitude and variability of the background inherent in the copper foil itself. Brightened copper foil not subjected to the deposition procedure in solution gives no measurable response on the chart. The same foil when processed in mercury-free impregnating solution gives a background varying from 0.06 to 0.09 square inches as shown in Table II.

#### Detector Calibration

In order to develop the relationship between the area under the curve and the quantity of mercury passed through the detector and thus to develop basic calibration data, known quantities of mercury metal were vaporized into the detector air stream. For this purpose mercury droplets of known diameter as determined by microscopic measurement are employed. The droplets are formed by smearing a microscope slide over a large mercury drop causing it to disintegrate into many tiny droplets. A droplet of suitable size is picked up by the point of a pin and placed on a clean microscope cover glass for measurement of diameter by conventional microscopic techniques prior to its vaporization in the detector air stream. Droplets as small as 30 to 40 microns can be handled in this manner.

The relation between area and mercury metal quantity is shown by the lower line of Figures 3 and 4. Earlier data showed a greater scatter which, it was discovered, resulted from a slight variation in instrument characteristics from day to day. Where high accuracy is required daily

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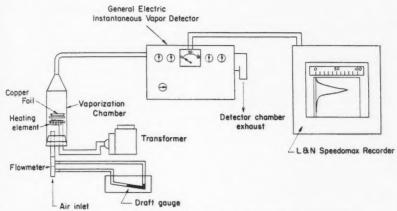


FIGURE 1. Equipment for analyzing mercury deposited on copper foil.

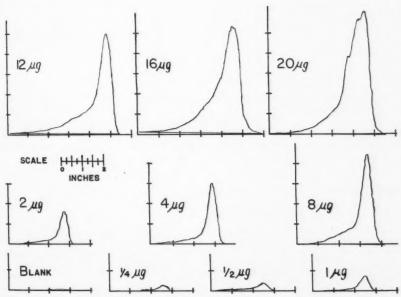


FIGURE 2. Typical detector response curves for various quantities of mercury.

calibration of the instrument by the procedure outlined above can be applied and will reduce the spread to a point where the maximum to minimum ratio is no greater than 1.2. A part of this inherent error is in the microscopic measurement of mercury droplets (about 1.1).

# Deposition Efficiency

To determine efficiency of deposition known quantities of mercuric chloride were placed on pieces of impregnated filter paper. These were placed in test tubes with water and copper foil following the standard procedure described previously. The area-quantity relationship is shown by the upper line of Figures 3 and 4. The ratio of these various values to corresponding points for mercury metal describe the deposition efficiency. The total data are summarized in Table III and show a gratifying consistency.

TABLE II

Magnitude and Reproducibility of Blank

Sample no.	Area (Sq in)
1	0.08
2	0.09
3	0.06
4	0.09
5	0.07
6	0.07

Average ...... 0.075

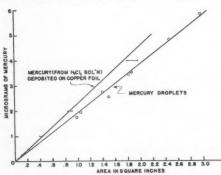


FIGURE 3. Calibration of G.E. Vapor Detector for mercury vaporized from copper foil.

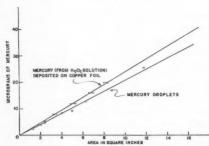


Figure 4. Calibration of G.E. Vapor Detector for mercury vaporized from copper foil. (Reduced scale.)

### Rate of Deposition

The elapsed time required for deposition of mercury in solution onto the copper foil is described in Figure 5. Deposition is approximately 65 per cent complete after the first 24 hours. Thereafter, the deposition rate decreases rapidly, as evidenced by the 80 per cent efficiency values after five days contact time. This still leaves 20 per cent unaccounted for. However, subsequent tests indicated that this residue might be totally accounted for by periodic addition of brightened copper foil during the contact period.

The lapse of time for increasing the recovery of mercury does not, it will be noted, involve any increased laboratory effort. On the other hand, if in particular circumstances the resulting delay be inconvenient, it would be permissible to limit the contact time to such a period as 16 to 24 hours and to apply the appropriate deposition efficiency factor in calculating results.

Table III
Deposition Efficiency of Mercury onto Copper Foil
after 6 Days Contact Time

No. of samples	Quantity of mercury, mg	Total area (sq in)	Deposition efficiency,
6	0	0.06-0.09	
4	1/4	0.18-0.19	86-94
3	3.6	0.28 - 0.29	82-86
4	1	0.47 - 0.50	79-84
3	2	0.91 - 0.98	82-88
3	4	1.86-2.03	88-96
4	8	3.23-3.50	78-84
3	12	4.89-5.41	84-92
3	16	6.62 - 7.09	86-92
3	20	8.06-8.46	83-87
1	40	17.01	88

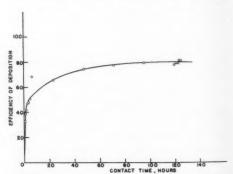


FIGURE 5. Rate of deposition of mercury onto copper foil.

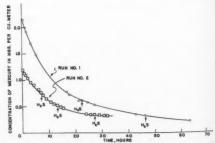


FIGURE 6. Effect of hydrogen sulfide on "die-away" rate of mercury vapor.

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# Effect of Hydrogen Sulfide on Mercury Vapor

We were interested to learn whether mercury vapor might react with hydrogen sulfide, a commonly occurring airborne contaminant. A study of this phenomenon was carried out in a 250cubic-foot chamber. A known quantity of mercury was vaporized in the chamber and the concentration periodically determined with the General Electric Vapor Detector. At specific time periods controlled quantities of hydrogen sulfide were introduced into the chamber with thorough mixing. Figure 6 shows the typical "die-away" curves for mercury vapor in this chamber. Hydrogen sulfide had no perceptible effect on the mercury vapor as indicated by the smoothness and continuity of the curves. If interference occurred, one would expect a downward break in the curve.

# Summary

A method has been described for the automatic sampling of mercury vapor, and for the estimation of quantities of mercury in such samples. The AISI smoke sampler supplied with paper impregnated with a potassium iodide reagent is employed for the sampling; a mercury vapor detector is used to measure the quantities of mercury in the samples. This method would have value in an industrial hygiene situation where automatic sampling at several locations would be desirable for a proper evaluation of a mercury hazard.

# Acknowledgments

This work was conducted by the writers while in the employ of the Industrial Hygiene Foundation at Mellon Institute; and was supported entirely by a grant from the American Petroleum Institute.

# Reference

 HAINES, GEORGE F., JR., WESLEY C. L. HEMEON, AND HERMAN CEMBER: Method for Tracing the Ground-Level Distribution of Stack Gas Emissions. J. Air Poll. Cont. Assoc. 7: 262 (February 1958).

# SHORT COURSES FOR ENGINEERS AND CHEMISTS

THE Division of Occupational Health, U. S. Public Health Service, announces two two-week courses on Industrial Hygiene Engineering and Industrial Hygiene Chemistry to be held at the Occupational Health Research and Training Facility, 1014 Broadway, Cincinnati 2, Ohio, beginning June 5, 1961. They will be followed by two one-week courses on Dust Evaluation Techniques and the Analysis of Silica, to be held concurrently from June 19 through 23, 1961. Applications should be addressed to the above Facility attention Chief, Training Operations Section. Sample schedules are available upon request.

Attendance in the general courses is limited to 12 engineers and 12 chemists. In the event that the courses are over-subscribed applicants that could not be accepted will be given preference for the repeat courses for Industrial Hygiene Engineers and Industrial Hygiene Chemists during the two-week period beginning October 9, 1961. Specialized courses will follow these sessions. Details will be included in the training program bulletin available from the Facility.

Applicants will receive a copy of the training manual and other literature pertaining to the course. No tuition is charged. Travel and living expenses must be borne by the student or his employer.

# A Light-scatter Probe for Aerosol Studies

# ROBERT L. DIMMICK, Ph.D.

Naval Biological Laboratory, School of Public Health, University of California, Berkeley, California

A back-angle light-scatter aerosol monitor is described which combines a light source and photocell in a cylindrical container. The unit may be inserted into an aerosol test chamber through a single hole. Sensitivity and recorded decay rate were found to be equivalent to results obtained with right-angle units, but the background light level may be high if the aerosol chamber is small or is not blackened on the inside.

CTUDIES of the physical properties of aero-Sols in the 1-10  $\mu$  particle diameter range are facilitated if a photoelectric aerosol monitor is located within the test chamber.1 In this arrangement samples are not withdrawn and the physical fall-out of the particulate matter is not disturbed. Further, a continuous record of aerosol presence can be obtained. However, if a lightscatter unit is to be installed in a test tank currently in use, some difficulty may be encountered in aligning the light path and optical configuration to conform to existing wall arrangements. It would be advantageous, therefore, if the light source and receptor were combined into a single probe-like unit which could be prealigned and inserted into the test tank through a single hole.

Figure 1 shows the essential features of a prototype unit which combined the light source and photomultiplier receptor in a tandem arrangement. The lamp housing and lens system, a simple lens projection unit which focuses the filament image about 2.5 cm beyond the forward diaphragm (C), were enclosed in a light- and air-tight tubular metal housing. The Westinghouse \$1492 lamp (D) was cooled by forcedair draft through inlet and exit tubes soldered to the housing. Since the spring contacts of the headlight replacement socket (E) varied in electrical resistance, wires were soldered directly to the lamp terminals, the contacts in the socket were removed, and the wires were dressed through these holes and through a piece of insulated tubing inserted in the plastic cone (G). The holes in (G) were made light-tight by metal grommets.

The light conduction system was made of plexiglass tubing, 4.1 cm o.d. and 3.5 cm i.d. and a piece of plexiglass rod 4.1 cm in diameter. The forward end of the tube was beveled at an angle of 20°. This produced a semi-lens shape with a focal length of approximately 5.0 cm. A funnel-shaped unit (G) was turned from the rod with the cone angle being 45° and the thickness of conical section 1.0 cm; the apex was turned to fit the entrance of an existing photocell housing (H). All light conducting or reflecting surfaces were polished. A metal endpiece (F) slipped over the cone and held it against the tube. All outer surfaces were given several coats of black paint and covered with black plastic tape. With this arrangement back-scattered light was conducted through the plastic cylinder and reflected from both angular surfaces of the cone onto the

To reduce background it was necessary to add the tube at (A) (a cone shape would have been more applicable) and the outer shield and diaphragm at (B). Before this change, the background was too high to read with the photometer used (Eldorado, Model PH-200, Eldorado Electronics, 2821 10th Street, Berkeley, California). Afterward the background was 0.05  $\mu$ a and was comparable to the 0.100  $\mu$ a observed with a right-angle scatter unit.

The instrument was tested in the chamber and by the method described in the reference using polystyrene latex (Dow, LS-063-A, 0.557  $\mu$  diam.). The particle sizes indicated by two experiments were 0.598 and 0.568  $\mu$ , respectively, with no correction for Cunningham's constant. The recorded physical decay curve, measured by the new instrument, duplicated that from one recorded concurrently by a right-angle unit monitoring the same aerosol and was within 5% of an identical sensitivity.

The principle of using a back-angle light-

This work was sponsored by the U. S. Army Chemical Corps, Fort Detrick, Frederick, Maryland, and the Office of Naval Research, U. S. Navy, under a contract between the Office of Naval Research and the Regents of the University of California. Reproduction in whole or in part is permitted for any purpose of the United States Government.

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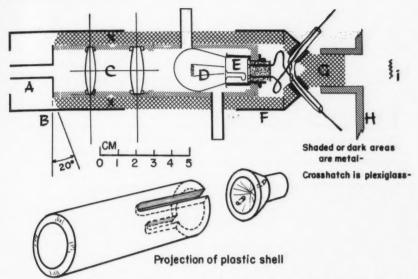


FIGURE 1. Details of the light-scatter probe. Cross section to scale. Bottom is view of configuration and assembly position of the plastic light conductor.

scatter unit, tubular in shape and capable of being inserted into almost any type of aerosol container, is shown to be practical and effective for observing aerosol decay.

#### Reference

 DIMMICK, R. L., M. T. HATCH AND J. NG: A Particlesizing Method for Aerosols and Fine Powders. AMA Arch. Ind. Health 18: 23 (July 1958).

### OBITUARY

Sigfrid Dahlberg, long time member of the American Industrial Hygiene Association, was one of the victims of the mid-air crash of two planes over New York on Friday, December 16, 1960. Mr. Dahlberg was consulting and research engineer for the American Fire Insurance Companies, and had been with them since 1935, when he joined the engineering department of the Fidelity and Casualty Company of New York. He was active with nuclear and atomic fission matters concerned with insurable risks.

Mr. Dahlberg, 63 years old, was a native of Sweden and a graduate of Guthenburg Polytechnic Institute and completed two years of post graduate study at Polytechnicum in Germany. He majored in electrical engineering and minored in chemistry, receiving his bachelour degree in 1919. Prior to coming to the United States he was with the Guthenburg Electric Utilities.

We join the many friends of Mr. Dahlberg in expressing our regrets and loss at his passing.

## ANNOUNCEMENTS AND INFORMATION

THE AMERICAN INDUSTRIAL HIGHER Association of the insertion of filler utilize the space on "short pages" at the ends of articles for the insertion of filler THE AMERICAN INDUSTRIAL HYGIENE Association Journal is pleased to items containing announcements and information of interest to industrial hygienists and others of our readers. We believe that announcements of meetings, conferences, symposia, courses, publications, and other exchanges of information are a real service to the industrial hygiene profession. Simple short informational items also serve in another manner. Such items are welcomed by the Editor.

Unfortunately many items, particularly announcements of meetings and courses, are received too late to be included in an issue appropriately in advance of the event. Details of such must reach the Editor about two months or more before the issue in which the items appropriately may appear. The Journal is issued in February, April, June, August, October, and December, and reaches the readers about the end of the month of issue. Accordingly, filler items for the respective issues must be prepared before the first of January, March, etc.

#### ORTHOPSYCHIATRIC MEETING

OPICS OF PARTICULAR INTEREST to specialists in industrial medicine and Loccupational health will be discussed at the thirty-eighth annual meeting of the American Orthopsychiatric Association at the Hotel Statler Hilton, New York City, March 22-25, 1961. The sessions will be attended by approximately 5,000 psychiatrists, psychologists, psychiatric social workers, educators, nurses and other specialists. Registration will be open to non-members as well as to members.

The Association brings together key disciplines involved in team approaches to prevention and treatment of behavior problems and related training and research.

The presidential session on March 23 will include an address by Renes Dubos, Ph.D., Professor at the Rockefeller Institute, on problems of biological adaptations of children to modern society. Other papers to be heard are: "Orthopsychiatry and Mental Health in Work Situations" by H. Meltzer, Ph.D.; "Team Approach to Developmental Treatment of Individuals in Industry" by William Zielonka, Ph.D.; and "Labors Approach to Mental Health in Industry" by Leo Perlis. For further information on the meeting and program write to Dr. Marion Langer, Executive Secretary, American Orthopsychiatric Association, 1790 Broadway, New York 19, New York.

